Group A: Project Management
A-1   Title and Approval Sheet

EPA Southeast New England Program (SNEP) for Coastal Watershed Restoration

Quality Assurance Project Plan (QAPP)

FOR EVALUATION OF NITROGEN REMOVAL EVALUATION PORTION OF STUDY ONLY
December 2016 – December 2019
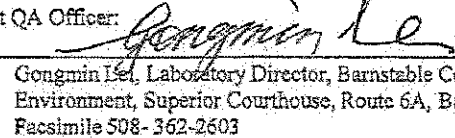As prepared by George Heufelder
Barnstable County Department of Health and Environment
November 15, 2016

Project Director:

_____   12/27/16
George Heufelder, Director, Massachusetts Alternative Septic System Test Center, a Division of Barnstable County Department of Health and Environment, Superior Courthouse, Route 6A, Barnstable, MA 02630; Phone 508-375-6616; Facsimile 508-362-2603

Project QA Officer: _____   12/27/2016
Gongmin Lei, Laboratory Director, Barnstable County Department of Health and Environment, Superior Courthouse, Route 6A, Barnstable, MA 02630; Phone 508-375-6606; Facsimile 508- 362-2603
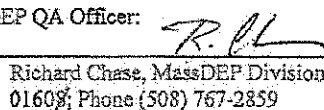
U.S. EPA QA Office Representative: _____   1/3/2017
Nora J. Conlon, Ph.D., U.S. EPA, Quality Assurance Unit, 11 Technology Drive, N. Chelmsford, MA 01863; Phone 617-918-8335

U.S. EPA Project Representative _____   12/27/2016
Bryan Dore, US EPA, Region 1 New England, 5 Post Office Square Boston, MA 02109-3912; Phone (617) 918-1211

MassDEP QA Officer: _____   12/27/16
Richard Chase, MassDEP Division of Watershed Management, 627 Main Street, Worcester, MA 01608; Phone (508) 767-2859   8 New Bond

Group A: Project Management
A-1 Title and Approval Sheet

EPA Southeast New England Program (SNEP) for Coastal Watershed Restoration

Quality Assurance Project Plan (QAPP)

FOR EVALUATION OF NITROGEN REMOVAL EVALUATION PORTION OF STUDY ONLY
December 2016 – December 2019
As prepared by George Heufelder
Barnstable County Department of Health and Environment
November 15, 2016

Project Director:

George Heufelder, Director, Massachusetts Alternative Septic System Test Center, a Division of Barnstable County Department of Health and Environment, Superior Courthouse, Route 6A, Barnstable, MA 02630; Phone 508-375-6616; Facsimile 508-362-2603

Project QA Officer:

Gongmin Lei, Laboratory Director, Barnstable County Department of Health and Environment, Superior Courthouse, Route 6A, Barnstable, MA 02630; Phone 508-375-6606; Facsimile 508- 362-2603

U.S. EPA QA Office Representative:

Nora J. Conlon, Ph.D., U.S. EPA, Quality Assurance Unit, 11 Technology Drive, N. Chelmsford, MA 01863; Phone 617-918-8335

U.S. EPA Project Representative

Bryan Dore, US EPA, Region 1 New England, 5 Post Office Square Boston, MA 02109-3912; Phone (617) 918-1211

MassDEP QA Officer:

Richard Chase, MassDEP Division of Watershed Management, 627 Main Street, Worcester, MA 01608; Phone (508) 767-2859

Contents

Appendix A:
Barnstable County Department of Health and the Environment Laboratory EPA Method 300.0 STANDARD OPERATING PROCEDURE for Determination of Inorganic Anions in Aqueous Samples Using Ion Chromatography (nitrate-nitrite).

Appendix B:
Barnstable County Department of Health and the Environment Laboratory EPA Method 350.1 STANDARD OPERATING PROCEDURE for Determination of Ammonia Nitrogen in Aqueous Samples by Semi-Automated Colorimetry

Appendix C:
Barnstable County Department of Health and the Environment Laboratory EPA Method 351.2 STANDARD OPERATING PROCEDURE for Determination of Total Kjeldahl Nitrogen in Aqueous Samples by Semi-Automated Colorimetry

Appendix D:
Barnstable County Department of Health and the Environment Laboratory SM 2540 D STANDARD OPERATING PROCEDURE for Determination of Total Suspended Solids in Aqueous Samples

Appendix E:
Barnstable County Department of Health and Environment Laboratory SM5210B STANDARD OPERATING PROCEDURE for the Determination of Biochemical Oxygen Demand (BODs) and Carbonaceous Biochemical Oxygen Demand (CBODs) in Aqueous Samples

Appendix F:
Barnstable County Department of Health and the Environment Laboratory SM 2320 STANDARD OPERATING PROCEDURE for Determination of Alkalinity in Aqueous Samples

Appendix G:
Barnstable County Department of Health and Environment STANDARD OPERATING PROCEDURE for use in field of YSI556 Dissolved Oxygen Meter/pH meter/temperature Calibrations.

Appendix H:
Barnstable County Department of Health and the Environment Laboratory SM 9222 D STANDARD OPERATING PROCEDURE for Determination of Fecal Coliform in Aqueous Samples.

George Heufelder, Director of the Massachusetts Alternative Septic System Test Center
Barnstable County Department of Health and Environment
3195 Main Street
Barnstable, Ma 02630 p: (508)-375-6616 gheufelder@barnstablecounty.org

Brian Baumgaertel, Public Health Sanitarian Environmental Specialist
Barnstable County Department of Health and Environment
3195 Main Street, Barnstable, Ma 02630 p: (508) 375-6888 bbaumgaertel@barnstablecounty.org

Gongmin Lei, Director
Barnstable County Department of Health and Environment Laboratory
3195 Main Street p: (508)-375-6606
Barnstable, Ma 02630   glei@barnstablecounty.org

Damann L. Anderson, P.E.
Vice President | Hazen and Sawyer
10002 Princess Palm Avenue, Suite 200, Tampa, FL, 33619
813 630-4498 (office) | 813 549-2116 (direct)
danderson@hazenandsawyer.com

Jose Amador, Ph.D.
Laboratory of Soil Ecology and Microbiology
024 Coastal Institute – Kingston
University of Rhode Island
Kingston, RI 02881 USA
Voice: 401 874 2902 | FAX: 401 874 4561
E-mail: jamador@uri.edu

George Loomis
Research and Extension Soil Scientist
Director, New England Onsite Wastewater Training Center
University of Rhode Island
001C Coastal Institute Bldg., NRS Dept.
1 Greenhouse Rd. Kingston, RI 02881
Voice: 401.874.4558 FAX: 401.874.4561
Email: GLoomis@uri.edu

Richard Chase
Mass DEP – Bureau of Water Resources, Division of Watershed Management, Watershed Planning Program
8 New Bond Street, Worcester, MA 01606   p: (508) 767-2859; f: (508) 791-4131
richard.f.chase@state.ma.us

Korrin Petersen, Esq.
Buzzards Bay Coalition
114 Front Street
New Bedford, MA 02740 p: (508) 999-6363 x 206
petersen@savebuzzardsbay.org

Bryan Dore, US EPA, Region 1 New England
5 Post Office Square Suite 100 Mail Code: OEP06-1
Boston, MA 02109-3912
Phone (617) 918-121
Dore.Bryan@epa.gov

Sarah Williams, Regional Planner.
Buzzards Bay National Estuary Program
2870 Cranberry Highway (Rt. 6)
East Wareham, MA 02538
Sarah.Williams@state.ma.us

A-4 Project Organization

The following describes the Project Organization for the collection and processing of samples collected under the EPA Southeast New England Program (SNEP) for Coastal Watershed Restoration.

```
                    ┌─────────────────────┐
                    │   Project Director   │
                    │   George Heufelder   │
                    └──────────┬──────────┘
          ┌────────────────────┼────────────────────┐
┌─────────────────┐  ┌─────────────────┐  ┌─────────────────────┐
│ Quality Assurance│  │ Field Sampling  │  │  Data Management    │
│   Gongmin Lei    │  │ George Heufelder│  │   and Reporting     │
└─────────────────┘  │ Brian Baumgaertel│  │  Brian Baumgaertel  │
                     │  Trained staff  │  └──────────┬──────────┘
                     └─────────────────┘             │
                                          ┌─────────────────────┐
                                          │  Data Check and     │
                                          │    Validation       │
                                          │ George Heufelder    │
                                          │   Gongmin Lei       │
                                          │ Brian Baumgaertel   │
                                          └─────────────────────┘
```
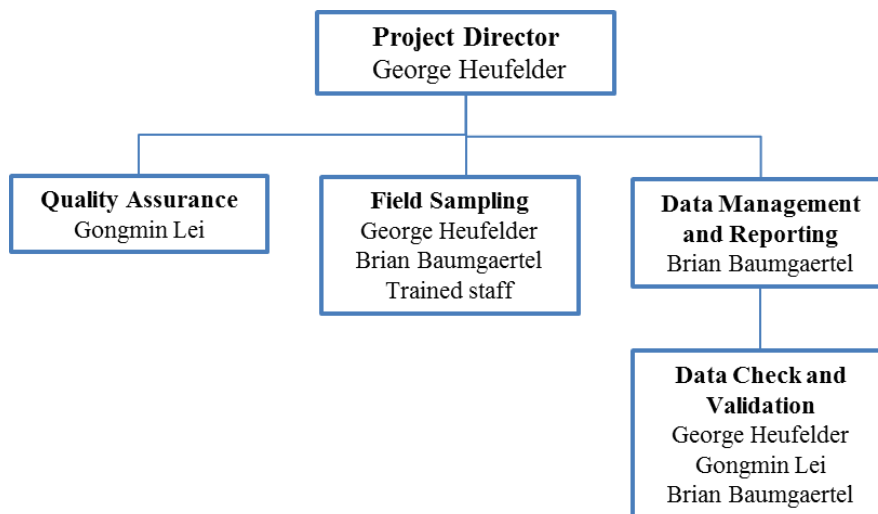
*Organization chart is only for that portion of the project involved in the collection and processing of chemical/biological analytes.

Samples to meet all regulatory requirements will be collected by staff of the Department of Health and Environment that will be trained by the staff below. A training log will be maintained to ensure that all collectors have been adequately trained in the collection and transport of samples and the use of the field instruments for the collection of data for pH, dissolved oxygen and temperature.

Only three primary staff are involved:

Massachusetts Alternative Septic System Test Center Director
George Heufelder, Barnstable County Department of Health and Environment
Superior Courthouse, Route 6A, Barnstable, MA 02630
Phone 508-375-6616; Facsimile 508-362-2603

1. Overall project oversight and planning.
2. Supervision of the sample collectors.
3. Financial planning and grant administration
4. Monitoring and operations QA/QC review
5. Data synthesis and report writing.

Public Health Sanitarian – Environmental Specialist
Brian Baumgaertel, Barnstable County Department of Health and Environment
Superior Courthouse, Route 6A, Barnstable, MA 02630
Phone 508-375-6888; Facsimile 508-362-2603

1. Collection of samples and training of collectors
2. Supervision of sample collectors
3. Data management
4. Data synthesis and report writing

Barnstable County Laboratory Manager
Gongmin Lei, Laboratory Director, Barnstable County Department of Health and the
Environment, Superior Courthouse, Route 6A, Barnstable, MA; Phone 508-375-6606

1. QA manager for the Barnstable County Laboratory
2. Oversees all operations of the laboratory
3. Reviews data from Barnstable County Laboratory and all subcontracted laboratories;
4. Coordinates all inquiries regarding data and the resolution of potential data problems by communication with other laboratory directors.

A-5   Problem Background and Problem Definition

The coastal areas of Southeast New England contain many embayments impacted by nitrogen that originates from onsite septic systems. In many areas, onsite septic systems will remain the dominant mode of wastewater disposal and treatment into the foreseeable future. While advanced treatment onsite septic systems provide a viable option to address the nutrient issue, their cost and complexity prevent their wholesale use in many instances. The project endeavors to demonstrate the potential for the reduction of nitrogen inputs from residential wastewater by employing a relatively passive non-proprietary modification of soil treatment area (STA), alternately known as leachfields or soil absorption systems.

A-6   Project Description

The Project involves the sampling of twelve onsite septic systems to be located based on homeowner preference and suitability of the site as determined by the George Loomis (University of Rhode Island), George Heufelder and the design engineer to determine the efficacy of these technologies for reducing nitrogen inputs from wastewater. The typical septic system is comprised of a septic tank followed by a STA that is either in sand meeting a certain specification or native soil. The modified STA studied herein is comprised of an 18" layer of a sand/sawdust situated directly beneath a layer of 18" of sand that complies with the fill requirements of 310 CMR 15.255(3)[1]. The efficacy of these systems will be assessed by comparing monthly samples of influent and effluent over two years and further comparing the performance with standard system STA.

Monitoring parameters

Monitoring parameters (Table 1) will be determined by the requirements of Massachusetts DEP testing protocols. Units undergoing testing or sampling will receive field measurements of temperature, pH, and dissolved oxygen in the effluent on the day of sample collection. All methodology for analyses is presented in Appendices A – F.

---

[1] 310 CMR 15.000: THE STATE ENVIRONMENTAL CODE, TITLE 5: STANDARD REQUIREMENTS FOR THE SITING, CONSTRUCTION, INSPECTION, UPGRADE AND EXPANSION OF ON-SITE SEWAGE TREATMENT AND DISPOSAL SYSTEMS AND FOR THE TRANSPORT AND DISPOSAL OF SEPTAGE

Table 1: Monitoring Parameters for the EPA Southeast New England Program (SNEP) for Coastal Watershed Restoration

Variable	Matrix	Units	Measurement Performance Criteria	Accuracy LFB (%)**	Accuracy LFM (%)**	Precision RPD (%)**
Alkalinity	Wastewater	mg/L as CaCO$_3$	See Appendix F:	94.8–104.1	N/A	≤ 12.6
Ammonium	Wastewater	mg/L	See Appendix B:	96–108	91–103	≤ 2.1
BOD or CBOD	Wastewater	mg/L	See Appendix E:	See Appendix E	See Appendix E	See Appendix E
Nitrate	Wastewater	mg/L	See Appendix A:	91–106	88–104	≤ 7.13
Nitrite	Wastewater	mg/L	See Appendix A:	95–106	83–108	≤ 3.40
Total Kjeldahl Nitrogen	Wastewater	mg/L	See Appendix C:	97–103	94–101	≤ 4.0
Total Suspended Solids	Water/Solids	mg/L	See Appendix D:	N/A	N/A	≤ 13.6

** LFB = Laboratory Fortified Blank; LFM = Laboratory Fortified Matrix; RPD = Relative Percent Difference.

A-7   Quality Objectives and Criteria for Measurement Data

The objective of the project is to compare the contaminant removal performance of a cellulose amended soil treatment unit (STA) with that of a standard unamended STA. Generally, performance of alternative septic systems is expressed as percent removal of a selected contaminant over a period of time. The actual performance of the system is gauged by the percent contaminant removal calculated by the following formula:

% Contaminant Removal = (1 – Effluent Concentration/Influent Concentration) X 100

In addition to reduction percentages, comparisons will be made between the amended and unamended STA using standard statistical tests depending on the distribution of the data and the adherence to the requirements of the test itself.

Precision

Precision is the degree of agreement among repeated measurements of the same characteristic, or parameter, and gives information about the consistency of methods. In context of this project, precision of measurements is a prime objective. It will be evaluated by comparison with laboratory precision objectives referenced in Table 1. Laboratory methods for measurement of precision are included in respective Standard Operating Procedures.

Data Representativeness

Data Representativeness is the extent to which measurements represent the true environmental condition. It is the degree to which data from the project accurately represent a technology being tested. Representativeness of samples is ensured by adherence to standard field sampling, measurement and laboratory protocols. The design of the sampling scheme and number of samples for this project provide representativeness of the performance of the technology being monitored. Representativeness of the data will be determined during data assessment and data interpretation phase.

Data Comparability

Data comparability is the degree to which data can be compared directly to similar studies. Comparability is ensured by using standardized sampling protocols and the same or comparable analytical methods and reporting with comparable sensitivity. Protocols used in this project are like those used in many other pilot projects and approval procedures for alternative septic system treatment performance.

Data Completeness

Data completeness is the comparison between the amounts of usable data collected versus the amount of data called for in the sampling plan. Completeness is the percentage of valid results obtained compared to the total number of samples taken for a parameter. The goal of this project is to meet or exceed 90% usable data.

A-8   Special Training/Certification

The Barnstable County Laboratory is certified by the Commonwealth of Massachusetts Department of Environmental Protection (Mass DEP) for analytes listed in Table 1. The Project Manager has a Massachusetts Wastewater Operator's License of Grade 4. All technical assistants will be required to complete the employee training checklist under direct supervision of the Project Manager (see Appendix G).

For each task relating to the collection and processing of sampling and the collection and recording of field (in situ) data, trainees will accompany the trainer personnel during a normal task-run. The trainer personnel will then observe the trainee during independent completion of a normal task-run to verify that all tasks have been performed correctly. The trainer personnel will then sign the training checklist record to verify that the trained personnel can perform the task correctly. Quarterly field audits will be conducted to verify proper taking and handling of samples as well as use of field instruments. All training records and audits will be maintained and included in appendices of the final report.

A-9   Documentation and Records

Data reports will be created at quarterly intervals with combined analytical results and operation and maintenance information. Analytical results will be in tabular format and will include data from field measurements and laboratory results. The Project Manager will receive the data from BCDHE laboratory QC data, and will compile the data reports. Laboratory turn-around-time is generally three weeks.

Observations of technology characteristics such as odor, noise, discharges and leaks, pauses in operation, equipment failure and any other observations which can be made by the facility operator will be logged in an operation and maintenance logbook. An operation and maintenance report will list these occurrences in tabular format. There is no prescribed standard for any of the technology characteristics listed above.

Quarterly data reports will be circulated to project principal investigators, QA officers and appropriate EPA personnel as listed in the distribution list (A-2) prior to release. A final report will be issued with evaluation of system performance for the non-proprietary technology within eight months of the end of the testing period.

Data will be retained by BCDHE for a period of 10 years.

Group B: Measurements and Data Acquisition

B-1   Sampling Process Design

Sample Sites and Site Selection

The project involves the installation of twelve septic systems at residences in the study area. These sites will be chosen based on two broad criteria; usage (seasonal or year-round) and soil type (outwash or moraine). All test sites will be located at residences where the water use can be documented. Other selection criteria include: distance to limiting conditions such as groundwater or impervious soils, distance from water resources, available space on the property to site a fully-sized system, results of a soil evaluation in accordance with Commonwealth of Massachusetts Regulations, willingness of the board of health to allow the piloting of the technology within their jurisdiction, willingness of the homeowner to allow frequent visits and monitoring and other minor criteria. Once a site is chosen and the soil treatment area (STA), alternately known as the soil absorption system or leachfield, is installed, four pan lysimeters will be installed to facilitate sampling as described below.

Sampling from the influent wastewater and the lysimeters described below is considered a composite representative sample.

Sample number and frequency

A minimum of three monthly samples will be taken at each site. One of these samples will be taken at the influent pump chamber (that is receiving septic tank effluent prior to its discharge to the soil treatment area or STA (alternately known as the leachfield or soil absorption system). The two additional samples will be taken from the following locations: one will be composited from two pan lysimeters collecting from the "treatment" STA (the one containing a cellulose-amended layer), and the other will be composited from two lysimeters placed in the "control" or unamended STA. These samples should allow an adequate comparison and evaluation of the effects of amendment.

Construction and sampling locations

One of two placement scenarios will be constructed at each site.

In the first scenario, a STA in compliance with the sizing requirements of 310 CMR 15.242 will be placed and enabled to receive septic tank effluent via a low pressure dosed pipe network. In addition, a STA sized for 50% of the design flow and having a layer of cellulose-amended material will be installed in various orientations beside the full-sized STA having no such amendment (Figure A).

During the piloting period, 50% of the design flow will be directed to the ½ of the STA designed in accordance with 310 CMR 15.000 (the control) and 50% of the design flow will be directed to the cellulose-amended STA (treatment). The placement and description of the pan lysimeters is described below.
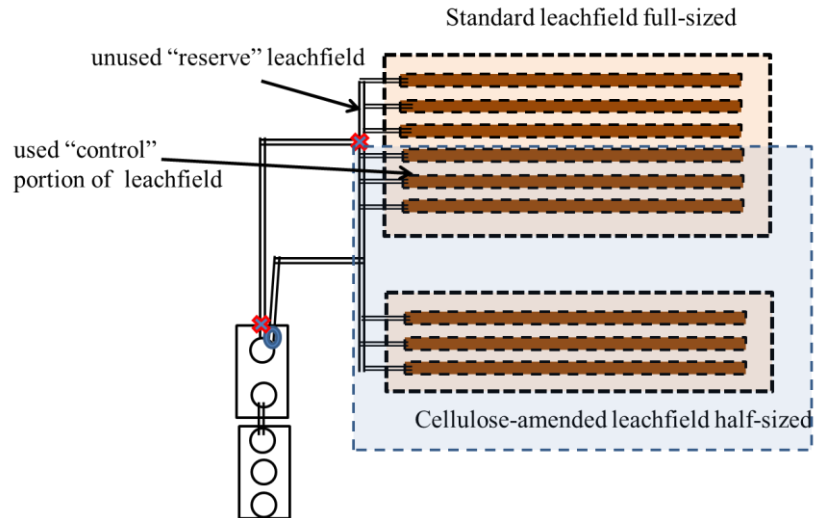
Figure A

In the second scenario, a STA in compliance with the sizing requirements of 310 CMR 15.242 will be constructed such that 50% of the system has a nominal spacing of the areal area of five feet from the other half. An amended cellulose layer will be placed beneath ½ of the STA and will constitute the "treatment" and the portion of the STA with sand meeting the fill requirements of 310 CMR 15.255(3) (Figure B).
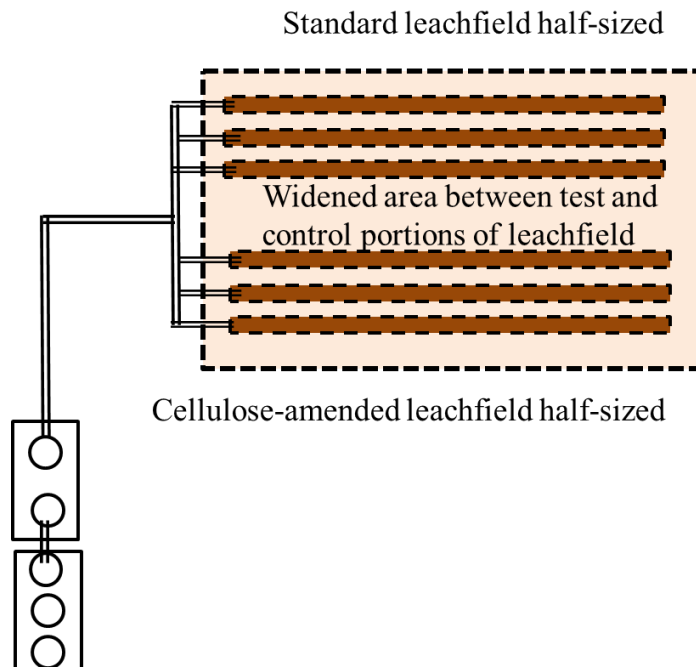
Figure B

Four pan lysimeters will be placed at each site. These lysimeters will be placed, two in each of the control section of the soil treatment units (STA) and the amended section of the STA. The pan lysimeter detail is provided in Figure C. The placement of the lysimeters along the STA is illustrated in Figure D.
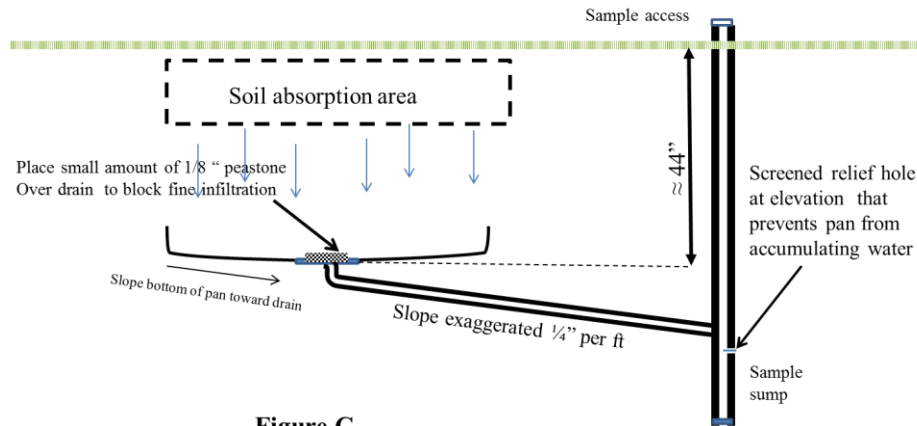
Figure C
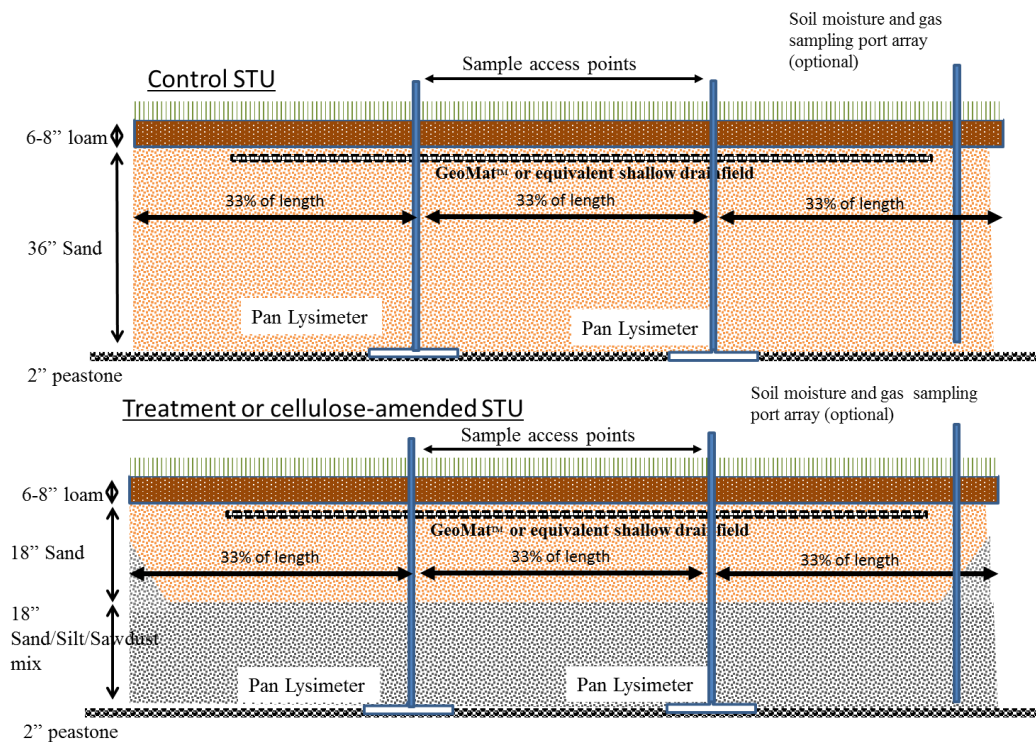
Figure D

Sample withdrawal procedure

Pan lysimeters will be sampled by means of a peristaltic pump. Initially all liquid in the sample sump will be withdrawn and voided. Following this a vacuum will be placed on the lysimeter for 60 seconds. Following another 60 seconds, a peristaltic pump is used to withdraw a sample. The Process is illustrated in Figure E.
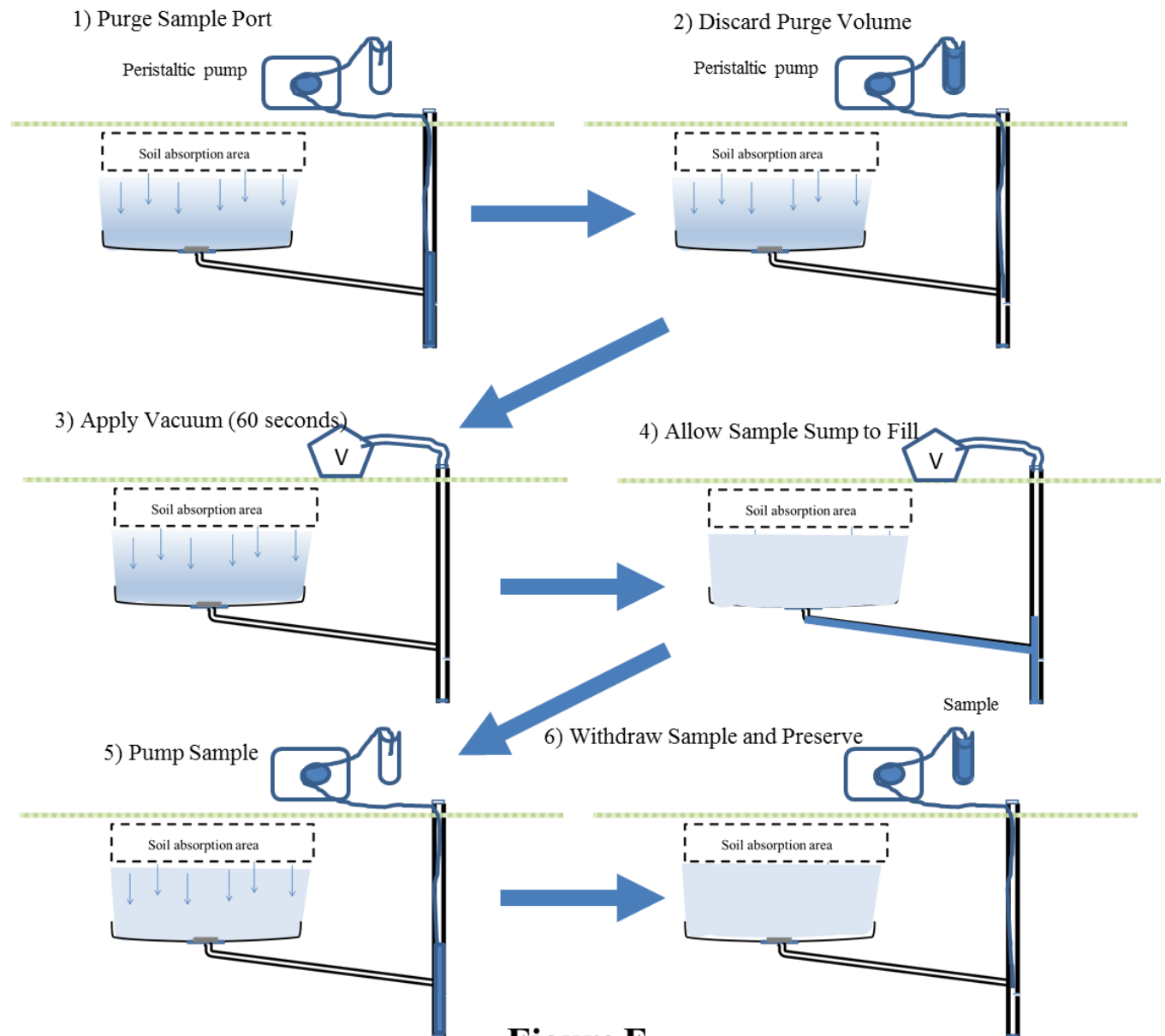
Figure E

Following sample withdrawal, both lysimeter samples will be combined to a single volume prior to pouring aliquots into appropriate containers. Samples will be preserved in appropriate containers and preserved and transported (See Table 2).

Influent Wastewater Sampling

Influent wastewater samples will be withdrawn from the facility pump chamber which follows the septic tank. A four-inch bailer will be used to fill sample containers. Sampling from this element of the septic system is considered as a composite sample of the prior 24 hours.

Sampling Parameters

The range of testing parameters is provided in Table 1.

Data analysis

The study described herein has a number of data objectives. One objective of this study is to evaluate the performance of the layered STA compared with other advanced onsite treatment technologies and strategies that have been installed in our geographical area for the removal of nitrogen. For this purpose, mean and median Total Nitrogen (TN = nitrate-nitrite + Total Kjeldahl Nitrogen) at the point of release to the environment (beneath the sand/sawdust mix layer) of the systems studies herein will be compared with point-of-discharge values obtained from a large database maintained by Barnstable County Department of Health and Environment. These comparisons will allow only for a coarse comparative evaluation of the layered STA.

The primary data objective will be the comparison of a standard STA installed in fill with the layered STA. Paired observations will be compared using appropriate statistical methodology. Analyte concentrations in percolate from beneath the sand/sawdust mix layer will be compared with percolate from a comparable elevation in the standard sand fill. With similar septic tank influent to both the treatment and standard STA, this will allow for a paired comparison of outcomes. While we cannot predict the characteristics of the data, a paired parametric test (Analysis of Variance) is the likely tool for this purpose. If the underlying assumptions of this statistical test are not met (i.e. similar variances in both the treatment and non-treatment units), we will employ non-parametric tests. Choice of statistical test will be guided by the EPA Document Data Quality Assessment: Statistical Methods for Practitioners EPA QA/G-9S.

In addition to the comparison of TN levels, regulatory officials will also likely with to know the comparison levels of Total Suspended Solids, Biochemical Oxygen Demand, ammonia and fecal coliform. With the exception of fecal coliform levels, we again predict that a comparison using parametric statistics will likely be indicated, while the fecal coliform data typically do not exhibit a normal distribution without a data transformation. Again, the above referenced EPA guidance will be used to determine the appropriate test for comparison.

B-2   Sampling Storage and Transport

Influent and percolate samples will be collected and stored in containers listed in Table 3. This table also lists holding times.

Measurement of temperature, dissolved oxygen and pH will be made from a one-liter aliquot of influent and percolate using the YSI 556 MPS (see Table 3 &4 and Appendix G for further specifications and calibration procedures/requirements).

Table 2. Containers used for analytes tested in the West Falmouth Harbor Project

Parameter	Container Volume	Container	Processing/Storage	Holding Time
Alkalinity	60-125 ml	Polyethylene	Stored on ice (dark)	14 Days
Ammonium	60-125 ml	Polyethylene (H_2SO_4 Preserved)	Stored on ice (dark)	28 Days
BOD or CBOD*	500 ml	Nalgene	Stored on ice (dark)	48 Hours
Nitrate + Nitrite	60-125 ml	Polyethylene	Stored on ice (dark)	48 Hours
Fecal coliform	100 ml	Plastic	Stored on ice (dark)	12 Hours
Total Kjeldahl Nitrogen	60-125 ml	Polyethylene (H_2SO_4 Preserved)	Stored on ice (dark)	28 Days
Total Suspended Solids	1000 ml	Polyethylene	Stored on Ice (dark)	7 Days

* It is required by NSF protocols that influent samples be tested for BOD and effluent samples be tested for CBOD.

Table 2 lists the frequency of measurement for each parameter in the two main classes of residences (seasonal and year-round).

Table 2. Frequency of testing various analytes.

	Year Round Homes influent	Year Round Homes Percolate beneath treatment	Year Round Homes Percolate beneath control
Total Suspended Solids	1	1	
Nitrite as Nitrogen	1	1	1
Ammonia as Nitrogen	1	1	
Nitrate as Nitrogen	1	1	1
TKN	1	1	1
Alkalinity	1	1	
Fecal Coliform (optional)	1	1	1
BOD, Carbonaceous	1	1	
Number of Samples per event	8	8	4
Number of Homes	6	6	6
Number of Events	24	24	24
	Seasonal Homes influent	Seasonal Homes Percolate beneath treatment	Seasonal Homes Percolate beneath control
Total Suspended Solids	1	1	
Nitrite as Nitrogen	1	1	1
Ammonia as Nitrogen	1	1	
Nitrate as Nitrogen	1	1	1
TKN	1	1	1
Alkalinity	1	1	
Fecal Coliform (optional)	1	1	1
BOD, Carbonaceous	1	1	
Number of Samples per event	8	8	4
Number of Homes	6	6	6
Number of Events	8	8	8

Table 3. Field measurements of wastewater

Parameter	Matrix	Units	Method	Reference
Temperature	Wastewater	℃	YSI 556 MPS	a
pH	Wastewater	pH	YSI 556 MPS	b
Dissolved Oxygen	Wastewater	mg/L	YSI 556 MPS	c

a. Temperature, Standard Methods 550
b. pH value, Standard Methods 4500-H$^+$
c. Dissolved Oxygen, Standard Methods 4500-O G. Membrane Electrode Method

Table 4. YSI 556 MPS Sensor Specifications

DISSOLVED OXYGEN	
Sensor Type:	Steady state polarographic
Range: *% air sat'n mg/L*	- 0 to 500% air saturation - 0 to 50 mg/L
Accuracy: *% air sat'n mg/L*	- 0 to 200% air saturation: ±2% of the reading or 2% air saturation; whichever is greater - 200 to 500% air saturation: ±6% of the reading - 0 to 20 mg/L: ±2% of the reading or 0.2 mg/L; whichever is greater - 20 to 50 mg/L: ±6% of the reading
Resolution: *% air sat'n mg/L*	- 0.1% air saturation - 0.01 mg/L
TEMPERATURE	
Sensor Type:	YSI Precision^{TM} thermistor
Range:	-5 to 45 ℃
Accuracy:	±0.15 ℃
Resolution:	0.01 ℃
pH	
Sensor Type:	Glass combination electrode
Range:	0 to 14 units
Accuracy:	±0.2 units
Resolution:	0.01 units

B-3   Sample Handling and Custody Requirements

Samples will be collected by trained field personnel with complete sample identification filled in on pre-formatted labels on each bottle. Sample identifications include the project name,

station, date, time, assay, and preservatives. Sample containers, labels, and chains of custody will be provided by BCDHE. Chemical preservatives are prepared in the laboratory prior to taking the sample in the field (TKN and ammonia samples).

Samples taken will be immediately placed in a cooler packed with ice or ice packs to maintain an internal temperature not to exceed 6 °C. Samples will be transported to the Barnstable County Department of Health and Environment Laboratory. All samples will be transported within a timeframe allowed by the holding times for each parameter. Chain of custody sheets will identify the sample number, technology, location, collection time, date, sampler, bottle type, and analysis required. Sample holding times are outlined in Table 2 (above).

B-4   Analytical Methods Requirements

Table 5. Laboratory Analysis

Laboratory Parameter	Method	Method Detection Limit**	Reporting Limit**
Alkalinity	SM 2320-B	0.46 mg/L	2.0 mg/L
Ammonium	SM 4500 NH$_3$C	0.23 mg/L	0.5 mg/L
BOD and CBOD*	SM 5210-B	2.0 mg/L	2.0 mg/L
Fecal Coliform (optional)	SM 9222D	1 CFU/100 ml	1 CFU/100 ml
Nitrate + Nitrite	EPA 300.0	0.070 mg/L	0.15 mg/L
Total Kjeldahl Nitrogen	SM 4500	0.23 mg/L	0.60 mg/L
Total Suspended Solids	SM 2540-D	3.1 mg/L	5.0 mg/L

* It is required by NSF protocols that influent samples be tested for BOD and effluent samples be tested for CBOD. Analyses for both parameters are conducted by Premier Laboratory.

**All MDLs and RLs reported here are accurate for the laboratory that conducts the analysis.

B-5   Quality Control Requirements

All quality control measures for analyses of samples taken can be found in the Barnstable County Laboratory Quality Assurance and Quality Control Plan, the Barnstable County Laboratory Standard Operating Procedures.

B-6   Instrument/Equipment Testing, Inspection, and Maintenance Requirements

Field Equipment:

All field measurements are taken by trained personnel. All instrumentation used in field measurement will be visually inspected prior to use to ensure proper operating condition. All observations of unusual occurrences, breakdowns, or malfunctions of the equipment will be recorded in the appropriate log book. During measurement, deficiencies in the accuracy of the instruments are often immediately apparent through the pre-and post-calibration procedures.

Acceptance, rejection, or qualification of field measurements will be based upon instrument calibrations. Field measurement QC data will be recorded by or be under control of the Project Manager. In all cases field calibrations and measurements will be reviewed in the field at the time of measurement. Rejection of a measurement could be due to excessive instrument drift or instrument malfunction, and would result in re- measurement or instrument re-calibration as necessary for validation.

Laboratory Equipment:
Refer to the Barnstable County Laboratory Quality Assurance and Quality Control Plan and Standard Operating Procedures for Barnstable County Laboratory.

B-7   Instrument Calibration and Frequency

Field Equipment:
1.   Thermometers will be checked against a reference thermometer prior to each round of sampling. Field thermometers will only be used if the difference between the field and reference thermometer is 1ºC or less. The reference thermometer shall be calibrated annually with a certified NIST thermometer to an accuracy of ± 1ºC. The NIST thermometer is quality checked annually by Alert Scientific. If there is a difference greater than one degree between the reference thermometer and the field thermometer, field personnel may use a backup thermometer (also NIST calibrated), or obtain a new thermometer for calibration from the Barnstable County Water Quality Laboratory. The results of each thermometer check are recorded in the thermometer logbook.

2.   The YSI 556 MPS (see Appendix G) will be calibrated daily prior to use as described by the manufacturer. Following use, calibration standards will be used to ensure that meter calibration was maintained during data collection. If the readings fall outside of the specified and allowable deviation from the standards (see Appendix G), then the meter will be recalibrated and all field measurements will be retaken. Standards used during calibration will be changed weekly. Results of the calibration, which include a beginning and end reading for temperature, dissolved oxygen, and a 7.00 pH standard will be recorded in the field logbook.

Laboratory Instrumentation:
Refer to the Barnstable County Laboratory Quality Assurance and Quality Control Plan and

Standard Operating Procedures for Barnstable County Laboratory.

B-8   Inspection/Acceptance of Supplies and Consumables

All supplies will be thoroughly inspected upon delivery and inventoried accordingly. Sample bottles will be stored on clean, dry, labeled shelves and checked for cracks or other forms of damage. Sample bottles treated with preservatives will be adequately marked and stored in an upright position. Sterile sample bottles for bacterial analyses will be inspected to ensure that they are undamaged and that the seal is intact.

All stock solutions used for calibration standards for instruments will be stored per manufacturer instructions. Portions used for daily calibrations will be replaced weekly. Stock standards solution will be marked with the date that they are opened and the expiration date, if applicable. Probes will be replaced when calibration readings fall outside of the specified and allowable deviation from the standards (see Appendix G). The temperature probe will be calibrated against a certified NIST thermometer.

B-9   Data Acquisition Requirements (Non-direct Measurements)

Water use meter readings will be obtained and recorded at each sampling visit.

B-10   Data Management

Hard copies of all sample results are obtained from Barnstable County laboratory on a regular basis. The sample data is manually entered into Microsoft Excel spreadsheets. Back-up copies of the Excel spreadsheets are made with CD-ROM. Hard copies of the sample reports, once entered into any appropriate spreadsheets, are filed. These files will be archived in the Barnstable County files for a minimum of 10 years.

Data Error Detection:

Detecting data errors will be accomplished by three methods: 1.) paper records of laboratory and field data will be compared with data entered in the database spreadsheets to detect errors of transcription; 2.) data will be graphed according to each measured parameter to check for outliers and other inconsistent data; 3.) graphical comparison of the measured parameters will be cross- checked for contradictory results. Data entry errors will be corrected as they are detected. Suspected errors in raw data will be referred to the relevant laboratory for further checks or for re-analysis of the sample, if within holding times. Unresolved data inconsistencies will be noted and reviewed for exclusion from the final dataset.

Group C: Assessment/Oversight

C-1   Assessments and Response Actions

The primary goal for the project is to develop equivalent or comparable data regarding the performance of both the standard and amended STA. Calibration of field instruments will take place prior to field sampling and calibration checks of the instruments will occur following every sample run. If calibration checks fail to fall within acceptable intervals for field instruments, measurements will be repeated following recalibration. During each run, at least one set of samples will be submitted blind to the laboratory. Data will be considered suspect and possibly subject to censoring if the duplicate sample falls outside of the precision level equal to two times the precision level indicated in Table 1 for laboratory duplicates. In that event, we will investigate the field variability of the waste stream by collecting five discrete samples in rapid succession. If the variability of these samples does not exceed two times the precision level indicated in Table 1 for laboratory duplicates, then the original data will be censored and not used in further statistical analyses of mean reductions.

External audits of the laboratory are regularly being performed by Mass DEP.

C-2   Reports to Management

Reporting of laboratory analyses will be conducted on an ongoing basis as the samples are processed and the reports are generated by the laboratories. Original reports will be delivered directly to the Project Manager.

If, during the compilation and presentation of reports, any data appear to be in error due to their anomalous value, the Project Director or Manager will request a re-inspection of the sample documentation and records and all records relative to the sample collection.

Group D: Data Validation and Usability

D-1   Data Review Verification and Validation

Data verification is conducted on at least three levels within the analytical laboratories for this project, with general oversight by the Project Q/A Officer (Director of the Barnstable County Department of Health and Environment).

Level 1 – Analyst review and verification:
All analysts comply with the standard operating procedures for each analyte. Sampling containers are checked for appropriate size, material and preservative as well as compliance with holding times and temperatures. Sampling methodology is verified for the matrix and source classification (wastewater, drinking water, etc.) and appropriate methods are employed based on these specifications. The chain of custody is checked and compared with analyte requests indicated on the sampling container. Data are accepted or rejected based on specifications for instrument or indicator performance specified in the standard operating procedures. All aberrations of instrument performance or test anomalies are reported directly to the Laboratory Director or immediate section supervisor for resolution.

Level two – Cross Review:

A review of data for each parameter entered into the laboratory database is conducted by a data reviewer. The individual who conducted the analyses or entered the data may not conduct the data review. At this point, the chain of custody is checked to make sure all analyses requested were completed and that the final results were correctly entered in the laboratory database.

Level three – Laboratory Director Review:

Prior to the release of data from the laboratory, the Laboratory Director checks the data for accuracy in reporting, assignment of proper reporting limits, and the attachment of all appropriate quality assurance documentation required by the customer. In addition, results are checked again for inconsistencies and errors. The Laboratory Director also checks that data produced by other subcontracted laboratories has been correctly transcribed.

Rejection of data will occur if the data fails to meet field or laboratory analytical QA/QC guidelines outlined in field and laboratory standard operating procedures. Other causes for rejection of data may result from system malfunctions, control equipment failures, power failures, or Acts of God which may directly affect the performance of a technology.

Field Measurements:

All field instrumentation is owned and controlled by BCDHE. Two identical instruments (YSI 556 MPS) are identically maintained and their use is alternated to ensure proper operation and instrument back-up. Acceptance, rejection or qualification of field measurements (dissolved oxygen, pH, and temperature) will be based upon the field QC methods; primarily instrument calibrations (see Appendix G). In all cases, QC data as well as field calibrations and measurement will be recorded and supervised by the project manager. Furthermore, a record of all calibrations and closing verification is maintained along with each day's measurements. Initial review of field-collected data will occur in the field, at the time of measurement. Rejection of a measurement could be due to excessive instrument drift or instrument malfunction, and would result in instrument re-calibration and re-measurement as necessary. In the event of instrument failure, the alternate instrument would be calibrated and measurements for that day would be re-taken.

Laboratory Measurements:

Additional QC requirements are outlined in the laboratory SOP and QA Plan. Rejection of data will be conducted by the Laboratory Director if there are breaches to the field or laboratory analytical QA/QC guidelines.

D-2   Validation and Verification Methods

Prior to submission to the Project Manager, laboratory data sets will have been reviewed first for completeness, errors in transcription, calculation, or computer input by the technical staff of the analytical labs. Laboratory analytical data will be submitted to the Project Manager in electronic and printed report format. Field and laboratory data will then be combined by the

Project Manager in a master spreadsheet and reviewed for tabular errors. Data will subsequently be viewed in graphical form for further review of outliers as possible errors. The data will also be reviewed for technical reasonableness, which is defined as: whether the data fails within known or expected limits of data previously generated or by other similar wastewater systems. Data which does not appear to be reasonable will be referred to the laboratory for review. Only data for which a definite miscalculation or transcription errors are documented will be changed. No data will be excluded, censored, or qualified. A note will be placed on all reports indicating the reason for exclusion of any data point from graphic representation or statistical analyses.

APPENDICES

Appendix A:

Barnstable County Department of Health and the Environment Laboratory EPA Method 300.0 STANDARD OPERATING PROCEDURE for Determination of Inorganic Anions in Aqueous Samples Using Ion Chromatography (nitrate-nitrite).

Appendix B:

Barnstable County Department of Health and the Environment Laboratory EPA Method 350.1 STANDARD OPERATING PROCEDURE for Determination of Ammonia Nitrogen in Aqueous Samples by Semi-Automated Colorimetry

Appendix C:

Barnstable County Department of Health and the Environment Laboratory EPA Method 351.2 STANDARD OPERATING PROCEDURE for Determination of Total Kjeldahl Nitrogen in Aqueous Samples by Semi-Automated Colorimetry

Appendix D:

Barnstable County Department of Health and the Environment Laboratory SM 2540 D STANDARD OPERATING PROCEDURE for Determination of Total Suspended Solids in Aqueous Samples

Appendix E:

Barnstable County Department of Health and Environment Laboratory SM5210B STANDARD OPERATING PROCEDURE for the Determination of Biochemical Oxygen Demand (BODs) and Carbonaceous Biochemical Oxygen Demand (CBODs) in Aqueous Samples

Appendix F:
Barnstable County Department of Health and the Environment Laboratory SM 2320 STANDARD OPERATING PROCEDURE for Determination of Alkalinity in Aqueous Samples

Appendix G:
Barnstable County Department of Health and Environment STANDARD OPERATING PROCEDURE for use in field of YSI556 Dissolved Oxygen Meter/pH meter/temperature Calibrations.

Appendix H:
Barnstable County Department of Health and the Environment Laboratory SM 9222 D STANDARD OPERATING PROCEDURE for Determination of Fecal Coliform in Aqueous Samples.

**Barnstable County Department of Health and the
Environment Laboratory**

EPA Method 300.0

STANDARD OPERATING PROCEDURE

For

Determination of Inorganic Anions in Aqueous Samples Using Ion Chromatography

Revision 011

November, 2015

		Signature	Date
Analysts:	Lacey Prior	*Lacey Adams Prior*	12/23/15
	Diane Brown	*Diane Brown*	12/23/15
Laboratory Director:	Gongmin Lei	*Gongmin Le*	12/28/2015

1

**Standard Operation Procedure for the Determination of Inorganic Anions
in Aqueous Samples Using Ion Chromatography**

1.0   SCOPE AND APPLICATION

1.1   This method covers the determination of the following inorganic anions in reagent waters, mixed domestic and industrial wastewaters, surface water, ground water, solids, leachates (when no acetic acid is used) and finished drinking water using ion chromatography.

Bromide, Chloride, Fluoride, Nitrate-N, Nitrite-N, ortho-Phosphate-P, Sulfate

1.2   This laboratory's Method Detection Limit (Section 8.1.2.1.) for the above analytes is listed in Table 1.

1.3   Whenever this method is used to analyze unfamiliar samples for any of the above listed anions, anion identification is supported by the use of a fortified sample matrix covering the anions of interest. The fortification procedure is described in Section 8.3.

2.0   SUMMARY OF METHOD

2.1   A 1.0 or 5.0 mL volume of sample is introduced into an ion chromatograph (IC). The anions of interest are separated and measured, using a system comprised of an ion chromatographic pump, sample injection valve, guard column, suppressor device, and a conductivity detector.

2.2   This method may be modified for limited performance-based attributes provided that they documented and meet the requirements expressed in the Quality Control Section (Section 8.0)

3.0   INTERFERENCES

3.1   Interferences can be caused by substances with retention times that are similar to and overlap those of the anions of interest. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution and/or fortification can be used to solve most interference problems associated with retention times.

3.2   The water dip or negative peak that elutes near, and can interfere with, the fluoride peak can usually be eliminated by addition of the equivalent of 1 ml of concentrated eluant (Section 6.2.1.) to 100 ml of each standard and sample

3.3   Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline ion chromatograms.

3.4 Any anion that is not retained by the column or slightly retained will elute in the area of fluoride and interfere. Known co-elution is caused by carbonate and other small organic anions. At concentrations of fluoride above 1.5 mg/L, this interference may not be significant, however it is the responsibility of the user to generate precision and accuracy information in each sample matrix.(Section 8.0, Quality Control.)

3.5 The acetate anion elutes early during the chromatographic run. The retention times of the anions also seem to differ when large amounts of acetate are present. This method is not recommended for leachates of solid samples when acetic acid is used for pH adjustment.

3.6 The quantitation of un-retained peaks should be avoided, such as low molecular weight organic acids (formate, acetate, propionate etc.) which are conductive and co-elute with or near fluoride and would bias the fluoride quantitation in some drinking and most waste waters.

3.7 Any residual chlorine dioxide present in the sample will result in the formation of additional chlorite prior to analysis. If any concentration of chlorine dioxide is suspected in the sample purge the sample with an inert gas (argon or nitrogen) for a minimum of 5 minutes until no chlorine dioxide remains.

4.0 SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are specifically listed below in Section 4.3 for hazardous materials.

4.2 The laboratory is maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) is available to all personnel involved in the analysis.

4.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

4.3.1 Sulfuric Acid (H_2SO_4), if and when used in preparation of the suppressant and when used a preservative. Protective eyeware, clothing and gloves should be worn when handling.

EQUIPMENT AND SUPPLIES

5.1 Ion chromatograph (IC): Dionex (Model ICS-2000; S/N 04020527)

This analytical system is complete with an EluGen II Potassium Hydroxide (KOH) Cartridge, an deionized (DI) water bottle with high purity of DI water (Resistivity >18.0 megohm-cm), an AS40 Automated Sampler, an ion chromatographic pump, injection valves, both guard and analytical separator columns, column heater, chemical suppressor, conductivity detector, and computer based data acquisition and process called CHROMELEON system (Dionex). Ion chromatograph

5.1.1 Anion guard columns – Dionex IonPac AG19, 2×50 mm (P/N 0037042). These guard columns function as a protector of the separator columns and packed with a substrate identical as that used in the corresponding separator column.

5.1.2 Anion separator columns. - Dionex IonPac AS19 Analytical column, 2×250 mm (P/N 062886). using the conditions outlined in Table 2.

5.1.3 Anion suppressor device – Dionex AERS 500 self-regenerating chemical suppressor (P/N 0082541). This built-in control for electrolytic Auto Suppressor eliminates the need to hand-prepare the acidic regenerant. Adequate baseline stability is attained as measured by a combined baseline drift/noise of no more than 0.5 nS per minute over the background conductivity.

5.1.4 Detector – Dionex Conductivity DS3 Detector.

5.1.5 ASDV Automated Sampler

5.1.6 AutoSampler Sample Vials – 0.5 ml vials equipped with filter caps (Dionex P/N 038010) or 5.0 ml vials equipped with filters (Dionex P/N 038141)

5.2. Data Acquisition System – The Dionex PEAKNET Data Chromatography Software was use to collect and generate all the data.

5.3 Analytical balance (±0.1 mg) – Fisher Scientific (Model ACCU-124D).

5.4 Top loading balance (±10 mg) – OHAUS (Model Scout II).

5.5 Syringes – Glass graduated syringes: 25 µL, 50 µL, 100 µL, 500 µL, 1000 µL.

5.6 Volumetric Pipets; Class A, 2, 4, 5 mL, 10 mL, 25 mL and 50 mL.

5.7 Eppendorf pipettor and tips

5.8 Volumetric Flasks – Class A, various volumes for preparing standards.

5.9 Sampling Containers – Glass or polyethylene, either purchased pre-cleaned or prepared in the laboratory. The containers should be of sufficient volumes to allow replicate analyses of anions of interest.

5.10 Water purification system (E-pure System) – Barnstead International (Model D4641 120 VAC).

5.11 Compressed Nitrogen Gas

5.12 Concentrated HCL – for glassware preparation in the use for oPhosphate-P analysis

6.0   REAGENTS AND STANDARDS

6.1   Reagent water – Deionized water from Direct QUV (Millipore Cat # ZRQSVP030): 18.0 Mohm or better. Or an equivalent Water Purification system.

6.2   Eluant – Dionex Eluent Generator Cartridge – Potassium Hydroxide (EGC III KOH) - Cat# 074532

6.3   Stock Standard Solutions
Stock standard solutions for the preparation of calibration standards, matrix spike solutions, LFB, QCS, are either purchased as certified solutions or prepared from ACS reagent grade materials as listed below.

6.3.1   Primary Source Stock standard solutions
The Primary source stock standard solutions are used for the preparation of calibration standards and are purchased from Inorganic Ventures as listed below:

	ppm	Catalogue #
Bromide (Br$^-$)	1000	ICBR1-1
Chloride (Cl-)	1000	ICCL1-5
Fluoride (F$^-$)	1000	ICFL-1
Nitrate as Nitrogen (NO$_3$-N)	1000	ICNNO31-1
Nitrite as Nitrogen (NO$_2$-N)	1000	ICNNO21-1
Phosphate as Phosphorus (PO$^=_4$-P)	1000	ICPPO41-1
Sulfate (SO$_4^=$)	1000	ICSO41-5

6.3.2   Secondary Source Stock standard solutions
These secondary source stock standard solutions are used for preparing the quality control check solutions (QCS). Any secondary stock solution chosen to be used for the QCS must be from a different manufacturing source or lot number that is being used as a primary source. These secondary source standards are either purchased as certified solutions or prepared from ACS reagent grade materials as listed below:

5

6.3.2.1 Purchased

Bromide (Br⁻) 1000 mg/L:
> Fisher - Cat# AS-BR9-2Ymm (100ml)
> VWR - Cat# EM 19896-1 (100 ml)
> UltraScientific - Cat# ICC-001 (100ml)

Chloride (Cl-) 1000 mg/L:
> VWR - Cat# EM ICA012-5 (500ml)
> UltraScientific - Cat# ICC007A (500 ml)

Fluoride (F⁻) 1000 mg/L :
> VWR - Cat# EM19814-1 (500 ml)
> UltraScientific - Cat# ICC-003 (100ml)

Nitrate as Nitrogen (NO_3-N) 1000 mg/L :
> VWR Cat# EMICA012-5 (500 ml)
> UltraScientific - Cat# ICC-004A (100 ml)

Nitrite as Nitrogen NO_2-N) 1000 mg/L :
> Fisher - Cat# AS-NO2N9-2Y (100 ml)
> UltraScientific - Cat# ICC-007A (100ml)

Phosphate as Phosphorus ($PO_4^=$-P) 1000 mg/L :
> Fisher -Cat# AS-PO4P9-2Y (100 ml)
> UltraScientific Cat# ICC-005A (100ml)

Sulfate ($SO_4^=$) 1000 mg/L:
> Fisher Cat# AS-SO49-2X (500ml)
> VWR - Cat# EM-198134-1 (500 ml)
> UltraScientific Cat# ICC-006-5 (500ml)

6.3.2.2. Prepared in Laboratory
> Prepared from purchased reagent grade chemicals that have been dried at
> 105oC for 30 minutes and cooled in dessicator prior to using:

Bromide (Br⁻) 1000 mg/L:

> Dissolve 0.6438g sodium bromide (NaBr,) in reagent water and dilute to 500 mL.
> (Baker Cat# 3588-01)

Chloride (Cl⁻)1000 mg/L:
> Dissolve 0.82425g sodium chloride (NaCl) in reagent water and dilute to 500
> mL.(Baker Cat# 3624-01)

Fluoride (F⁻) 1000 mg/L:
>Dissolve 1.1050g sodium fluoride (NaF,) in reagent water and dilute to 500 mL. (Baker Cat# 3688-01)

Nitrate as Nitrogen (NO₃-N) 1000 mg/L:
>Dissolve 3.03395 g sodium nitrate (NaNO₃,) in reagent water and dilute to 500 mL. (Baker Cat# 713108)

Nitrite as Nitrogen (NO₂-N) 1000 mg/L:
>Dissolve 2.46285g sodium nitrite (Na NO₂,) in reagent water and dilute to 500 mL. (EMScience Cat# SX0665-1)

Phosphate as Phosphorus (PO⁼₄-P) 1000 mg/L:
>Dissolve 2.19685g   potassium phosphate (KH₂PO₄,) in reagent water and dilute to 500mL. (EMScience Cat# PX1565-1)

Sulfate (SO₄⁼) 1000 mg/L:
>Dissolve 0.6438g potassium sulfate (K₂SO₄) in reagent water and dilute to 500 mL. (Sigma Cat# P-4167)

6.4   Once standards are purchased and received, or prepared, they are logged in the Primary   Standard Logbook with date of receipt, name of vendor, catalog number, expiration date and a primary standard ID assigned. Purchased chemicals with Certificate of Analyses provided by the vendor will have the laboratory assigned primary standard ID, date and the receiving analyst initials. The bottle will also be identified with primary standard ID and the date received and the analyst initials.

An example of the Logbook is attached (Figure 1).

Primary standard ID is labeled as IP mmddyy X:

Where: IP = Inorganic Primary

mmddyy = the date the standard is received

X = the order that the standard is logged into the logbook on that date in increasing alphabetical order.

6.5   Preparation of Calibration Standards – For each analyte of interest, intermediate calibration standards are prepared by first adding measured volumes of one or more stock standards (Section 6.3.1.) to volumetric flasks and diluting to volume with reagent water. These intermediate calibration standards are then further used to prepare the working calibration standards. This laboratory separates the calibrations into the following analytes to be determined within a sample run.

6.5.1   Fluoride, NitriteN, NitrateN - Combined

   6.5.1.1   Intermediate Calibration Standard - Fluoride, NitriteN, NitrateN -
             Combined the following aliquots of stock solutions are combined together in a
             100 ml volumetric flask.

        a   5 mL of 1000 ppm Fluoride stock standard (Section 6.3.1.) to yield 50
                mg/L Fluoride.
        b.   5 mL of 1000 ppm Nitrite-N stock standard (Section 6.3.1.) to yield
                50 mg/L Nitrite as Nitrogen.
        c.   10 mL of 1000 ppm Nitrate-N stock standard (Section 6.3.1.) to yield 100
                mg/L Nitrate as Nitrogen.

   6.5.1.2 Working Calibration Standards – a minimum of 5 levels are needed for
            construction a curve.

            There are eight concentration levels for the calibration curve for F, Cl, NO2-
            N, NO3-N, SO4 as follows :

ppm	Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
Level 1	0.050	1.00	0.05	0.10	1.00
Level 2	0.125	2.50	0.125	0.25	2.50
Level 3	0.25	5.00	0.25	0.50	5.00
Level 4	0.50	10.0	0.50	1.00	10.0
Level 5	1.25	25.0	1.00	2.50	25.0
Level 6	2.50	50.0	2.50	5.00	50.0
Level 7	5.00	100	5.00	10.0	100
Level 8	10.0	200	10.0	20.0	200

            The working standards are prepared via serial dilutions starting with the combined
            Cl, NO2-N, NO3-N, SO4 Intermediate Standard (Section 6.5.1.1)

            Level 8 – 20 ml of 1000 ppm Chloride & Sulfate, 20 ml Intermediate Combined
                       F, NO2,& NO3-N to 100 ml
            Level 7 – 20 ml of 1000 ppm Chloride & Sulfate, 20 ml Intermediate Combined
                       F, NO2-N & NO3-N to 200 ml
            Level 6 – 10 ml of 1000 ppm Chloride & Sulfate, 10 ml Intermediate Combined
                       F, NO2-N & NO3-N to 200 ml
            Level 5 – 5 ml of 1000 ppm Chloride & Sulfate, 5 ml Intermediate Combined F,
                       NO2-N & NO3-N to 200 ml
            Level 4 – 10.0 ml of Level 7 to 100 ml
            Level 3 – 5.0 ml of Level 7 to 100 ml
            Level 2 – 2.5 ml of Level 7 to 100 ml
            Level 1 – 2.5 ml of Level 7 to 200 ml

6.5.2   o-PO4-P

    6.5.3.1   Intermediate Calibration Standard.
          5 mL of 1000 ppm oPO$_4$-P stock standard (Section 6.3.1) in a 500 ml
          volumetric flask to yield 10.0 mg/L oPO$_4$-P.

    6.5.2.2   Working Calibration Standards
          There are six concentration levels for the calibration curve for oPO$_4$-P
          as follows :

	oPO$_4$-P (ppm)
Level 1	0.05
Level 2	0.10
Level 3	0.25
Level 4	0.50
Level 5	1.00
Level 6	2.50

          The working standards are prepared via serial dilutions starting with the
          oPO$_4$-P Intermediate Standard.

          Level 6 - 25 ml of Intermediate Standard to 100 ml
          Level 5 – 5.0 ml of Intermediate Standard to 50 ml
          Level 4 – 5.0 ml of Intermediate Standard to 100 ml
          Level 3 - 5.0 ml of Level 6 to 50 ml
          Level 2 – 5.0 ml of Level 5 to 50 ml
          Level 1 – 5.0 ml of Level 4 to 50 ml

6.5.3   Bromide

    6.5.3.1   Intermediate Calibration Standard -
          10 mL of 1000 ppm Bromide stock standard (Section 6.3.1.) in a 100 ml
          volumetric flask to yield 100 mg/L. Bromide

    6.5.3.2   Working Calibration Standards
          There are seven concentration levels for the calibration curve for Bromide as
          follows (with Level 1 as the reporting limit concentration)

	Bromide (ppm)
Level 1	1.0
Level 2	2.5
Level 3	5.0

Level 4   10.0
Level 5   25.0
Level 6   50.0
Level 7   100.0

The working standards are prepared via serial dilutions starting Intermediate Standard.

Level 7 – Intermediate Standard
Level 6 – 25.0 ml of Intermediate Standard to 50 ml
Level 5 – 25.0 ml of Intermediate Standard to 100 ml
Level 4 – 10.0 ml of Intermediate Standard to 100 ml
Level 3 – 5.0 ml of Intermediate Standard to 100 ml
Level 2 – 2.5 ml of Intermediate Standard to 100 ml
Level 1 – 1.0 ml of Intermediate Standard to 100 ml

6.6   After the working calibration standards are made they are logged in the Working Standard Logbook with date of preparation, initial concentration, amount taken, final volume, final concentration, solvent used, expiration date , analysts initials and assigned an Working Standard ID (see Figure 2).

Working standard ID is labeled as IW mmddyy X:

Where: IW = Inorganic Working

Mmddyy = the date the standard is made.

X = the order that the standard is made on that date in increasing alphabetical order.

6.7   Preparation of Quality Control Check (QCS) solutions
These quality control check solutions are prepared using the secondary source stock standard solutions (Section 6.3.2) to verify new calibration curves and continual verification on a quarterly basis.

6.7.1   Fluoride, Nitite-N, Nitrate-N – Combined Intermediate Standard

6.7.1.1 Intermediate Standard
Combined the following aliquots of stock solutions are combined together in a 100 ml volumetric flask.

a   5 mL of 1000 ppm Fluoride stock standard (Section 6.3.1.) to yield 50 mg/L Fluoride.
b.   5 mL of 1000 ppm Nitrite-N stock standard (Section 6.3.1.) to yield 50 mg/L Nitrite as Nitrogen.
c.   10 mL of 1000 ppm Nitrate-N stock standard (Section 6.3.1.) to yield 100 mg/L Nitrate as Nitrogen.

Prepare as a Level 7 (Section 6.5.1.2.) - 20 ml of 1000 ppm Chloride & Sulfate, and 20 ml Intermediate Combined F, NO2-N & NO3-N to 100 ml

6.7.3 o-PO4-P

6.7.3.1 Intermediate Standard.
5 mL of 1000 ppm oPO$_4$-P stock standard (Section 6.3.2.) is pipetted into in a 100 ml volumetric flask and diluted to the mark with deionized water to yield 50.0 ppm solution.

6.7.3.2 From this intermediate standard the QCS is prepared by pipetting a 2 mL aliquot into a 200 mL and diluted to the mark with deionized water to yield a 0.50 ppm solution

6.7.4 Bromide

6.7.4.1 5 mL of 1000 ppm Bromide stock standard (Section 6.3.2.) pipetted into in a 100 ml volumetric flask to yield 50 mg/L.

6.8 Preparation of Instrument Performance Check Solution (IPC)
These quality control check solutions are prepared using the primary source stock standard solutions (Section 6.3.1) at the mid-range concentrations of the calibration curve and is used to verify the curve on an on-going basis during the sample sequence run.

6.8.1. Prepare as Level 6 (Section 6.5.1.2.) – 10 ml of 1000 ppm Chloride & Sulfate, 10 ml Intermediate Combined Standard: F, NO2-N & NO3-N to 200 ml

6.8.1.1. Alternate procedure – Using 1000 ppm Stock Solutions pipette the following aliquots into 1000 ml volumetric flask and brought to mark with deionized water.

Fluoride – 2.5 mL
Chloride - 50 mL
Nitrite-N - 2.5 mL
Nitrate-N - 5.0 mL
Sulfate - 50 mL

6.9 Preparation of Laboratory Fortified Blanks (LFB)

6.9.1 Prepare as a Level 5 (Section 6.5.1.2.) 5 ml of 1000 ppm Chloride & Sulfate, 5 ml Intermediate Combined Standard F, NO2-N & NO3-N to 200 ml

6.9.1.1 Alternate procedure – Using 1000 ppm Stock Solutions pipette the following aliquots into 1000 ml volumetric flask and brought to mark with deionized water.

Fluoride – 1.25 mL
Chloride - 25 mL
Nitrite-N – 1.25 mL
Nitrate-N – 2.5 mL
Sulfate - 25 mL

6.9.2   o-PO4-P

Using the Intermediate Calibration Standard (Section 6.5.3.1.) pipette 5 ml into a 200 mL volumetric flask and dilute to the mark with deionized water. This yields a 0.5 ppm LFB solution.

6.9.3   Bromide

Using the Intermediate Calibration Standard (Section 6.5.4.1.) pipette 25 ml into a 100 mL volumetric flask and dilute to the mark with deionized water. This yields a 25 ppm LFB solution

6.10   Preparation of Matrix Spike (MS) solution used for fortifying samples

6.10.1 Fluoride,Chloride, Nitrite-N, Nitrate-N, Sulfate - Combined

Using 1000 ppm Stock Solutions pipette the following aliquots into 1000 ml volumetric flask and brought to mark with deionized water.

Fluoride – 50 mL
Chloride - 50 mL
Nitrite-N - 2.5 mL
Nitrate-N - 5.0 mL
Sulfate - 50 mL

Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
2.50	50.0	2.50	5.0	50.0 ppm

6.10.2 o-PO4-P

Using the Intermediate Calibration Standard (Section 6.5.3.1.) pipette 10 ml into a 200 mL volumetric flask and dilute to the mark with deionized water. This yields a 1.0 ppm matrix spike solution

6.10.3 Bromide

5 mL of 1000 ppm Bromide stock standard (Section 6.3.2.) pipetted into in a 100 ml volumetric flask to yield 50 mg/L.

6.11   Preparation of Low Level Check Standard (LLC)

6.11.1 Fluoride, Chloride, Nitrite-N, Nitrate-N, Sulfate
Using the Level 4 combined Calibration Standard (Section 6.5.1), pipette 2.5 ml to a 200 mL volumetric flask and dilute to the mark with deionized water. This yields a LLC solution of the following concentrations:

Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate
0.05	1.00	0.05	0.10	1.00 ppm

6.11.2 o-PO4-P
Using the Intermediate Calibration Standard (Section 6.5.3.1.) pipette 0.5 ml into a 200 mL volumetric flask and dilute to the mark with deionized water. This yields a 0.50 ppm LLC solution.

6.11.3 Bromide
Using the Intermediate Calibration Standard (Section 6.5.4.1.) pipette 1.0 ml into a 100 mL volumetric flask and dilute to the mark with deionized water. This yields a 1.0 ppm LLC solution

7.0   SAMPLE COLLECTION, PRESERVATION AND STORAGE

7.1   Samples are collected in plastic or glass bottles that have been either purchased pre-cleaned or prepared in the laboratory by thoroughly cleaning and rinsing bottles sufficient with reagent water (Section 5.5.). The volume collected is sufficient to insure a representative sample and allow for replicate analysis and fortification if necessary.

7.2   Samples are shipped iced or stored cold in a cooler at ≤ 4.0 °C. The laboratory will not accept samples whenever the sample bottle has been violated (i.e. loose or broken cap, leaking bottle, improperly labeled), causing concern for contamination.

7.3   Following are the sample preservation and holding times :

Analyte	Preservation	Holding Time
Bromide	None Required	28 days
Chloride	None Required	28 days
Fluoride	None Required	28 days
Nitrate-N	Cool to 4.0 °C	48 hours
Nitrite-N	Cool to 4.0 °C	48 hours
Combined Nitrate/Nitrite*	conc. H_2SO_4 to pH <2	28 days
o-Phosphate-P	Cool to 4.0 °C	48 hours
Sulfate	Cool to 4.0 °C	28 days

*Note: If the determined value for the combined nitrate/nitrite exceeds 0.5 mg/L as N, a resample must be analyzed for the individual concentrations of nitrate & nitrite.

7.4   Allow any cooled sample to come to room temperature before analysis. In the case of ortho-phosphate it has been observed that degradation occurs in samples that have been held at room temperature for over 16 hrs.

8.0   QUALITY CONTROL

Consists of an initial demonstration of laboratory capability and the on-going assessment of the quality of the data being generated by analysis of laboratory reagent blanks, fortified blanks, quality control samples, and the determination of analyte recoveries. The generated performance records are kept on file and available for review for ten years in accordance this laboratory's QA/QC plan.

8.1   INITIAL DEMONSTRATION OF PERFORMANCE - Refer to Table 4

8.1.1   The instrument's performance and the laboratory's performance is assessed prior to conducting any analyses. The Instrumentation Performance is characterized via the determination of Linear Calibration Range (LCR) and analysis of Quality Control Samples (QCS) The laboratory performance is characterized via the determination of MDL's. (see Table 1).

8.1.2   Linear Calibration Range (LCR) – The LCR is determined initially The verification of linearity uses a blank and a minimum of three standards prepared in the following concentrations listed as below. If the verification data exceeds the initial values by $\pm$ 10%, linearity is re-established. Any non-linear portion of the defined range is nonlinear, then additional standards are used to define the nonlinear portion. Refer to Section 6.5. for the preparation of the Calibration Standards.

8.1.3   Quality Control Sample (QCS) – When first beginning this method, the calibration standards and instrumentation performance is verified by analyzing a QCS from a second source. If the determined concentration are not within $\pm 10\%$ of the expected values, performance of the determinative step of the method is unacceptable. The source of the problem is identified and corrected before proceeding with the initial determination of MDL's

8.1.4.   Method Detection Limit (MDL) – MDL's are established for all analytes using reagent water (blank) fortified at concentrations of two-to-three times the estimated instrumentation detection limit. To determine the MDL values, seven replicate aliquots of the fortified reagent water are analyzed and concentrations determined over a period a minimum of 3 days. The fortified concentrations and preparation procedures used for the analytes are listed as follows:

Analyte	mg/L	Procedure Section
Bromide	1.00	Section 6.5.4 - Level 1
Chloride	1.00	Section 6.5.1 - Level 1
Fluoride	0.25	Section 6.5.3 - Level 2

Nitrate-N	0.10	Section 6.5.1 - Level 1
Nitrite-N	0.05	Section 6.5.2 - Level 1
o-Phosphate-P	0.05	Section 6.5.2 - Level 1
Sulfate	1.00	Section 6.5.1 - Level 1

For each analyte , calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where:

t = Student's value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]

S = standard deviation of the replicate analyses

MDL's are determined every at least every six months or whenever a significant change in the background or instrument response is detected or expected and kept on file for 10 years. (See example in Table 1).

8.2   ASSESSING LABORATORY PERFORMANCE

8.2.1   Accuracy & Precision Studies (A&P)

Annually, the accuracy & precision of each element is determined.

To establish this accuracy & precision for each element, a minimum of seven replicate analyses of a mid-range Calibration Standard is analyzed.

Use the listed Calibration Standards concentrations for the A&P's studies

Anion	ppm	Level Standard	See Section
Bromide	50	Level 6	Section 6.5.4.2.
Chloride	50	Level 6	Section 6.5.1.2.
Fluoride	5.0	Level 6	Section 6.5.2.2.
Nitrate-N	5.0	Level 6	Section 6.5.1.2.
Nitrite-N	2.5	Level 6	Section 6.5.1.2.
o-Phosphate-P	1.0	Level 5	Section 6.5.3.2.
Sulfate	50	Level 6	Section 6.5.1.2.

The accuracy for each analyte is measured by determining the % Recovery of the seven results using the following calculation :

$$\%REC = \frac{(C_s - C)}{S} \times 100$$

Where:
    % REC = percent recovery,
    C_s = average of the seven determinations

15

C   = concentration of prepared analyte

The Precision for each analyte is expressed as the standard deviation estimate with n-1 degrees of freedom of the seven replicate results and kept on file for 10 years (See example in Table 2)

8.2.2   Method Detection Limits (MDL)
Annually, and every six months or whenever a significant change in the background or instrument response is detected or expected the MDL's are established for all analytes.
To determine the MDL values see Section 8.1.4.

8.2.3.   Analyte Recovery and Data Quality – Refer to Table 5
On an on-going basis, the laboratory's performance is continually assessed.

8.2.3.1 Laboratory Fortified Blank (LFB) - At least one LFB is analyzed with each batch of 20 samples. The accuracy is calculated as percent recovery (Section 8.4.2.). If the recovery of any analyte falls outside the required control limits of 90-110%, then that analyte is considered to be out of control and the source of the problem is determined and resolved before continuing analyses. Following are the concentrations of the LFB (preparation procedure : see Section listed below).

Analyte	mg/L	Procedure Section- conc. Level
Bromide	25.0	Section 6.5.4.2 - Level 5
Chloride	25.0	Section 6.5.1.2 - Level 5
Fluoride	2.50	Section 6.5.2.2 - Level 5
Nitrate-N	2.50	Section 6.5.1.2 - Level 5
Nitrite-N	1.25	Section 6.5.1.2 - Level 5
o-Phosphate-P	1.00	Section 6.5.3.2 - Level 5
Sulfate	25.0	Section 6.5.1.2 - Level 5

8.2.3.1.1. Calculation of Percent Recoveries - calculate the percent recovery for each analyte, corrected for concentration measured in the unfortified sample. These values are compared to the determined LFM recovery range of 90-110-%.

The percent recovery is calculated as follows:

$$\%REC = \frac{Cm}{C} \times 100$$

where,
% REC = percent recovery,
C_m = measured fortified sample concentration,
C = prepared fortified sample concentration,

16

8.2.3.1.2   The LFB analyses data is used to assess the laboratory's performance against the required control limits of 90-110%. When enough internal performance data is available (minimum of 25 analyses) control limits are established for each analyte. These upper and lower control limits are determined from the percent mean recovery (x) and the standard deviation (S) and are established as follows :

$$\text{UPPER CONTROL LIMIT} = x + 3S$$

$$\text{LOWER CONTROL LIMIT} = x - 3S$$

These control limits must be equal to or better than the required control limits of 90-110%. After each 5-10 new recovery measurements, new control limits are calculated on the most recent 25 data points.

In addition, the standard deviation (S) data is used to establish an on-going performance statement for the level of concentrations included in the LFB. These data are kept on file and are available for review.

8.2.3.1.3   These results are incorporated into the on-going Control Charts to document data quality as outlined in Section 8.3 and are available for review for 10 years.

8.2.3.2.   Laboratory Fortified Sample Matrix (LFM) – The laboratory adds a known amount of the analyte to a minimum of 10% of the collected field samples or at least one with every analysis batch, whichever is greater. This is accomplished by adding equal volumes of the sample to be fortified with an equal amount of the following concentrations then followed by pouring and the necessary portion of such to be analyzed. The concentration of each analyte added is as follows with the preparation procedure section as listed:

Anion	MDL x4*	mg/L MS	Final Conc	Procedure Section
Bromide	0.4	50	25	Section 6.9.4
Chloride	4.0	50	25	Section 6.9.1
Fluoride	0.4	5.0	2.5	Section 6.9.2
Nitrate-N	0.4	5.0	2.5	Section 6.9.1
Nitrite-N	0.2	2.5	1.25	Section 6.9.2
o-Phosphate-P	0.2	1.0	0.5	Section 6.9.3
Sulfate	4.0	50	25	Section 6.9.1

In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The added analyte concentration is the same that is used in the laboratory fortified blank (The analyte concentration must be high enough to be detected above the original sample and not less than four times the MDL*). If the concentration of the fortification is less than 25% of the background concentration of the matrix the matrix recovery is not calculated.

8.2.3.2.2. Calculation of Percent Recoveries - calculate the percent recovery for each analyte, corrected for concentration measured in the unfortified sample. These values are compared to the determined LFM recovery range of 90-110- %.

The percent recovery is calculated as follows:

$$\%REC = \frac{(C_s - C)}{S} \times 100$$

Where :
    % REC = percent recovery,
    C_s = measured in the fortified sample,
    C = measured sample concentration,
    S = concentration equivalent of analyte added to sample.

Until sufficient becomes available (minimum of 20 analysis) assess the laboratory performance against recovery limits of 80-120%. When sufficient data becomes available develop control limits from percent mean recovery and the standard deviation of the mean recovery.

8.2.3.2.3. If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that all other QC performance criteria is acceptable, the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related.
Repeated failure to meet suggested recovery criteria indicates potential problems with the procedure and will be investigated.

8.2.3.2.4. These results are incorporated into the on-going Control Charts to document data quality as outlined in Section 8.3 and are available for review for 10 years

8.2.3.3 Laboratory Sample Duplicates – The laboratory analyzes sample duplicate for a minimum of 10% of the collected samples or at least one with every analysis batch, whichever is greater. These results are incorporated to the on-going control charts to document data quality.

Calculate the relative percent difference (RPD) of the initial quantitated concentration (I_c) and duplicate quantitated concentration (D_c) using the following formula

$$RPD = \frac{|(I_C - D_c)|}{([I_C + D_c]/2)} \times 100$$

Where:

RPD = Relative Percent Difference

I_c = initial quantitated concentration

Dc = duplicated quantitated concentration

Duplicate analysis may exhibit matrix dependence. If the RPD for the duplicate measurements falls outside ± 20% and if all other QC performance criteria are met, laboratory precision is out of control for the sample and perhaps the analytical batch. The result for the sample and duplicate will be labeled as suspect/matrix to inform the data user that the result is suspect due to a potential matrix effect, which led to poor precision. This should not be a chronic problem and if it frequently recurs (>20% of duplicate analyses), it indicates a problem with the instrument or individual technique that must be corrected.

8.2.3.4. Laboratory Fortified Blank Duplicates - Quarterly, replicates of the LFB's are analyzed to determine the precision of the laboratory measurements. The RPD is determined as outlined above in Section 8.2.3.3. These results are incorporated to the on-going duplicate (precision range) control charts to document data quality.

8.2.4   QC CONTROL CHARTS

Two types of control charts are used for the continued assessment of the lab's performance :
    (1) Accuracy , or Means, Control Chart
    (2) Precision , or Range, Control Chart

8.2.4.1   The Accuracy Chart is constructed using the most recent 25 LFB and sample MS %Recovery results. See Section 8.2.4.3 for Calculation of %Recoveries for the LFB and Section 8.2.4.6 for the MS. The upper and lower warning limits (WL) use ± 2SD and the upper and lower control limits (CL) use ± 3 SD .

8.2.4.2   The Precision Chart is constructed using the most recent 25 Sample & Sample Duplicate RPD results. See Section 8.2.4.7 for the calculation of RPD. The warning limits (WL) use ± 2SD and the control limits (CL) use ± 3 SD.

8.2.4.3. Application of Control Charts.

8.2.4.3.1.   Trending – If seven successive samples are on the same side of the central line of the Accuracy Chart, discontinue analyses, investigate and correct the problem

8.2.4.3.2.   Control Limit – If one measurement exceeds a CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses, if it exceeds the CL, discontinue analyses, investigate and correct the problem.

8.2.4.3.3.   Warning Limit – If two out three successive points exceed a WL, analyze another sample. If the next point is within WL, continue analyses.
If the next point exceeds the WL, evaluate potential bias and correct the problem.

8.2.5 The following items must be included in every sample batch or periodically to continually assess the laboratory's performance. See Table 5. A batch of samples is   established as 20 samples:

Calibration Curve - verified every quarterly with QCS or whenever a significant change in instrument response is detected or expected. See Section 6.1. for the preparation of the calibration standards.

Instrument Blank (IB) – to verify system clear of residual artifacts & contaminants

Instrument Performance Check Solution (IPC)- a mid-range check standard after calibration, every 10 samples and at end of sample sequence run

Laboratory Reagent Blank (LRB) – after IPC in beginning, every 10 samples   after CCS, and at end of sample sequence run.

Laboratory Fortified Blank (LFB) – one per batch of samples (every 20 samples)

Laboratory Fortified Sample Matrix (LFM) – a minimum of 10 % of sample sequence run.

Sample duplicates – a minimum of 10% of sample run

LFB Duplicates - Quarterly

Low Level Check Standard (LLC) - Quarterly

MDL's – every 6 months

8.2.5.1 Instrument Performance Check Solution (IPC) – The laboratory analyzes the IPC (a mid-range check standard) of the following concentrations after the Instrument   & Calibration blanks are run at the beginning of the day's sample sequence, after every tenth sample and at the end of the sample run. The procedures for preparing the IPC is listed under   the listed sections.

Analyte	mg/L	Procedure Section
Bromide	50.0	Section 6.5.4 - Level 6
Chloride	50.0	Section 6.5.1 - Level 6
Fluoride	5.0	Section 6.5.2 - Level 6
Nitrate-N	5.0	Section 6.5.1 - Level 6
Nitrite-N	2.5	Section 6.5.1 - Level 6
o-Phosphate-P	1.0	Section 6.5.3 - Level 5
Sulfate	50	Section 6.5.1 - Level 6

Subsequent analyses of the IPC must verify that the calibration is still within $\pm 10\%$. If the calibration cannot be verified within the specified limits, reanalyze the IPC. If the second analysis of the IPC confirms the calibration to be outside the limits, sample analysis must

be stopped, the cause determined. All samples following the last acceptable IPC must be reanalyzed.

8.2.5.2 Laboratory Reagent Blank (LRB) – An LRB is prepared and treated exactly as a typical field sample including exposure to all glassware, equipment, solvents, filtration and reagents that are used with field samples. Data produced are used to assess contamination from the laboratory environment.
Values that exceed the MDL indicate a laboratory or reagent contamination is present. The source of the contamination must be determined prior to conducting any sample analysis.
Any sample included in an automated analysis batch which has an invalid LRB, indicated by a quantitated result that exceeds the MDL, must be reanalyzed in a subsequent analysis batch after the contamination problem is resolved.

8.2.5.3 Laboratory Fortified Blank (LFB) – Refer to Sections 8.2.4.3 & 6.4. for preparation procedure.

8.2.5.4 Laboratory Fortified Sample Matrix (LFM) – Refer to Sections 8.2.4.6 & 6.9. for preparation procedure.

8.2.5.5 Sample Duplicates – Refer to Section 8.2.4.7.

8.2.5.6 Low Level Check (LLC) – Quarterly, the lowest level standard (MDL) is analyzed to demonstrate the ability to analyze low level samples. Refer to Section 6.4 for preparation procedure.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Establish ion chromatographic operating parameters indicated in Table 2.

9.2 Run the initial calibration using the standards made in Section 6.5. Using injections of 25 microliters (determined by the injection loop volume) of each prepared calibration standard.

9.2.1 The initial calibration is deemed acceptable if the following criteria are met (Table 4):
$R \geq 0.9950$

9.3 The calibration curve is verified by analyzing a IPC immediately after the initial calibration, every 10 samples and at end of sample sequence run.

9.3.1 The concentration of the IPC used for the separate analytes are as follows. The procedure for making these standards are the same as those from making the indicated concentration levels of the initial calibration standards (Sections 6.5) but from a separate (secondary) source as those stock solutions as used in the making of the calibration standards

Analyte	mg/L	Procedure Section
Bromide	50	Section 6.5.4.2

21

Chloride	50	Section 6.5.1.2
Fluoride	5.0	Section 6.5.2.2
Nitrate-N	5.0	Section 6.5.1.2
Nitrite-N	2.5	Section 6.5.1.2
o-Phosphate-P	1.0	Section 6.5.3.2
Sulfate	50	Section 6.5.1.2

9.3.1.2 The IPC concentration must fall within ± 10% of the stated value. If the response or retention time for any analyte varies from the expected values by more than ±10%, the test is repeated, using fresh IPC standards. If the results are still more than ± 10 %, a new calibration curve must be prepared for that analyte.

9.4 End of Run IPC- at the end of the sample run sequence

9.5 End of Run Blank – at the very end run of the day – an instrumentation blank is run using reagent deionized water

10.0 <u>PROCEDURE</u>

10.1 Samples Preparation

10.1.1 See Section 7.3 for sample storage & handling conditions. Those samples that require refrigeration, ensure the samples have come to room temperature prior to conducting sample analysis.

10.1.2 Samples Pretreatment - The pretreatments prescribed are effective at reducing the chloride and sulfate content of a sample matrix but will not reduce matrix concentrations of other anions such as nitrate or phosphate

10.1.2.1 If the Chloride concentration interferes with the determination of NO2 or NO3 then pre-treat the sample using a Ag pretreatment cartridges to remove the Chloride (Dionex P/N 057089).

10.1.2.2 If the Sulfate concentration interferes with the determination of oPO4 then pre-treat the sample using Ba pretreatment cartridges to remove the sulfate (Dionex P/N 057093).

10.1.2.3 Samples Pretreatment Procedure
Individually and thoroughly rinse each pretreatment cartridge with reagent water in order to insure all residual background contaminants are removed from the cartridge. Filter 3 mL of sample through the series of rinsed cartridges as an initial sample rinse (Ba, Ag) at a flow rate of 1.0 mL/min or less (approximately one drop every 3 to 4 seconds). This flow rate is critical to the pretreatment and must be carefully followed. Discard this fraction and begin collecting the pretreated sample aliquot of collected sample.

10.1.2.4 Pour approximately 0.75 ml sample into 0.5ml autosampler vail (or 6 ml into 5 ml autosampler vail) and place a filter cap into the vial and push down the cap with a special made tool from Dionex to certain position according to instructions provided by the Manufacturer. There is no need to filter the sample since the cap has a filter in it.

10.1.3 Prior to pretreating any field samples, prepare and pretreat both an LRB and an LFB. These pretreated quality control samples are required when an analysis batch contains a matrix that must be pretreated. The pretreated LRB and LFB are used to verify that no background interference or bias is contributed by the pretreatment. If a response is observed in the pretreated LRB, triple or quadruple the volume of reagent water rinse used and repeat until a blank measures no more than ½ the MRL. If this additional rinsing procedure is required, it must be consistently applied to all the cartridges prior to conducting any matrix pretreatment.

10.1.4 Solid Samples - The following extraction should be used for solid materials. Add an amount of reagent water equal to 10 times the weight of dry solid material taken as a sample. This slurry is mixed for 10 minutes using a magnetic stirring device. Filter the resulting slurry using a 0.45u membrane type filter. Ensure that good recovery and peak identification is obtained through the use of fortified samples.

10.2. Sample Analysis

10.2.1. Table 2 summarizes the operating conditions for the ion chromatograph. Included in this table are a representative retention time and MDL results for the analytes that has been achieved by this method

10.2.2 Verify the initial calibration by conducting a QCS. (Section 8.4 -the QCS is prepared in the laboratory using stock solutions obtained from a secondary source - either purchased or prepared from reagent grade chemicals- Section 6.3.2.)

10.2.3 The injection volume is 25 microliters that is controlled by using 25 microliters sample loop (Dionex P/N: 052682). Use the same size loop for standards and samples. An AS40 Automated Sampler (Dionex P/N: 056830) is used. Data acquisition and processing are done using CHROMELEON CHM-1-IC/Win 2000 Desktop Workstation (Dionex P/N: 060929).

10.2.4 The retention time window used to make identifications in the laboratory is ± 0.2 minutes (determined by ±3 Std Dev of the RT of individual analytes over the course of a day)

10.2.5 If the response of a sample analyte exceeds the calibration range, the sample is diluted with an appropriate amount of reagent water and reanalyzed.

10.2.6 If the resulting chromatogram fails to produce adequate resolution, or if identification of specific anions is questionable, fortify the sample with an appropriate amount of standard and reanalyze.

10.2.7 An analytical sequence including initial calibration and other quality control analysis for sample analysis is listed in Table 5.

11.0 DATA ANALYSIS, CALCULATIONS AND REPORTS

11.1 Identify the analytes in the sample chromatogram by comparing the retention time of a suspect peak within the retention time window to the actual retention time of a known analyte peak in a calibration standard. The retention time in the daily calibration check standards (QCS) is used for the identification.

11.2 Compute sample concentration using the initial calibration curve generated in Section 8.1.

11.3 Report those values that fall between the MRL and the highest calibration standards without any flagging. Sample analytes with responses that exceeds the highest calibration standard concentration are diluted and reanalyzed.

11.4 A printout of the sample sequence is printed out, dated & initialed, and kept in a notebook (Example of sequence run copy is attached – Table 5). Hard copies of the integrated analyses are printed and kept in filing folder indentified by the sequence number.

11.5 Report results in mg/L. The MRL reported is the lowest Calibration Standard Level used

11.6 Report : NO_2^- as Nitrogen
NO_3^- as Nitrogen
HPO_4 as P

12.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

The laboratory waste management practices are conducted consistent with all applicable rules and regulations as stated in the laboratory's "Sample and Waste Disposal Standard Operating Procedure" , Revision 003 - July 6, 2006). Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

13.0 REFERENCES

1. U.S. Environmental Protection Agency, "Determination of Inorganic Anions by Ion Chromatography", Method 300.0, Revision 2.1, August 1993

2. Standard Methods for the Examination of Water and Wastewater, Method 4110B, "Anions by Ion Chromatography", 22th Edition of Standard Methods (2012)

Table 1. Method Detection Limits (MDLs)

MDL Study	Year 2013					
Analyte	Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate	Bromide
Spiking Level (ppm)	0.100	1.00	0.050	0.100	1.00	1.00
Run #1	0.188	1.12	0.0581	0.171	0.697	0.962
Run #2	0.130	1.12	0.0579	0.166	0.697	0.964
Run #3	0.119	1.12	0.0584	0.166	0.714	0.962
Run #4	0.147	1.12	0.0579	0.171	0.717	0.958
Run #5	0.120	1.12	0.0568	0.165	0.719	0.952
Run #6	0.119	1.16	0.0531	0.173	0.703	0.966
Run #7	0.117	1.12	0.0549	0.165	0.700	0.974
Average	0.124	1.126	0.057	0.168	0.708	0.962
1 Std Dev	0.011	0.016	0.002	0.003	0.012	0.007
MDL	0.034	0.051	0.006	0.011	0.037	0.021
Reporting MDL	0.10	0.10	0.050	0.10	1.0	1.0
Dates Run	08/13/13	07/26/13	07/29/13	07/26/13	07/26/13	09/17/13
	08/14/13	07/29/13	07/30/13	07/29/13	07/29/13	09/19/13
	08/16/13	07/30/13	07/31/13	07/30/13	07/30/13	09/19/13
Analyst	L.Prior	L.Prior	L.Prior	L.Prior	L.Prior	L.Prior

Table 2. Accuracy and Precision (A&P)

A &P Study	Year 2013					
Analyte	Fluoride	Chloride	Nitrite-N	Nitrate-N	Sulfate	Bromide
Spiking Level (ppm)	5.00	12.5	2.50	2.50	12.50	50.0
Run #1	4.958	13.95	2.845	2.510	14.60	49.69
Run #2	4.960	13.99	2.778	2.512	14.64	49.55
Run #3	4.960	13.99	2.848	2.513	14.66	49.32
Run #4	4.967	14.04	2.841	2.536	14.67	49.42
Run #5	4.958	14.07	2.88	2.530	14.67	49.59
Run #6	4.966	14.02	2.881	2.520	14.67	49.41
Run #7	4.963	14.01	2.884	2.523	14.78	49.57
Average	4.962	14.01	2.851	2.521	14.67	49.51
% RSD	0.004	0.039	0.037	0.010	0.055	0.128
% Mean	99.2	112.1	114.0	100.8	117.4	99.0
Analysis Dates	08/13/13	07/26/13	07/29/13	07/26/13	07/26/13	09/17/13
Analyst	L.Prior	L.Prior	L.Prior	L.Prior	L.Prior	L.Prior

Table 3.   Chromatographic Conditions and Equipment of the Ion Chromatographic Instrument

Ion Chromatograph:	Dionex ICS-2000
Sample Loop:	25 µL
Eluent:	EGC III KOH @ 22.0 mM
Eluent Flow:	0.23 mL/min
Columns:	Dionex IonPac AG19 Guard Column 2×50 mm Dionex IonPac AS19 Analytical column, 2×250 mm
Typical System Backpressure:	1900 psi
Suppressor:	Dionex AERS 500 self-regenerating chemical suppressor @ 16 mA current
Detector:	Dionex DS6 - Detection Stabilizer Conductivity at 16 mA held at a temperature of 30°C . Background Conductivity: 0.2 – 1.0 µs
Total Running Time:	15 minutes

Table 4. Initial Demonstration of Capability and Acceptance Requirements

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.1.1.1	Initial Linear Calibration Range (LCR)	Generate calibration curve. At least 5 calibration standards are recommended.	MRL must be no lower than the lowest calibration standard. $R \geq 0.9950$
Section 8.1.1.2.	Quality Control Sample	An external/second source of analyte standard must be run following the initial calibration.	The QCS must be $\pm$ 10% of the true value.
Section 8.1.2.	Method Detection Limit (MDL) Determination	Analyze 7 replicate LFBs of the lowest Calibration Standard Level over a period of three days minimum. MDL is determined based on these results.	
Section 8.2.1.	Initial Demonstration of Accuracy and Precision	Analyze 7 replicate LFBs fortified with analyte. Calculate the mean recovered concentration ($C_{\overline{x}}$) and the relative standard deviation (%RSD).	The $C_{\overline{x}}$ must be $\pm$ 10% of the true value, and the %RSD must be $\leq$ 10%.
Section 8.2.3	Minimum Reporting Level (MRL)	MRL = Chloride, Sulfate = 1.0 mg/L, Nitrate-N,=0.10 mg/L, Nitrite-N= 0.05 mg/L, Fluoride =0.50mg/L, Bromide=1.0 mg/L	The low CAL standard can be lower than the MRL, but the MRL must be no lower than the low CAL standard.

Table 5.   Quality Control Requirements

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.2.2.	Calibration Curve	At least 5 calibration standards are recommended. New Curves should be established every six months or whenever significant changes have occurred. If Retention Times's drift more than 10% - establish a new curve	MRL must be no lower than the lowest calibration standard. $R \geq 0.9950$
Section 8.2.3.	Initial IPC	Analyze after Instrument and Method blanks	Recoveries must be between 90-110% of fortified level.
Section 8.2.2.1	Continuing IPC and Ending IPC	Analyze after 10 samples and after the last sample in an analysis batch.	Recoveries must fall between 90-110%.
Section 8.2.2.2	Laboratory Reagent Blank (LRB)	Analyze at the beginning, after 20 samples and after the last sample in an analysis batch	The LRB concentration must be $\leq$ the proposed MDL.
Section 8.2.2.3.	Laboratory Fortified Blank (LFB)	Analyzed with each batch of samples (20 or less).	Recoveries must be between 90-110% of fortified level
Section 8.2.2.4	Low Level Check (LLC)	Analyzed Quarterly	Recoveries must be between 70-130% of fortified level
Section 8.2.2.5.	Laboratory Fortified Sample Matrix (LFM)	Must add known amount of analyte to a minimum of 10% of field samples or at least one within each analysis batch.	Recovery must be 80-120%. If fortified sample fails the recovery criteria, label both as suspect/matrix.
Section 8.2.2.9.	Field or Laboratory Duplicates	Analyze either a field or laboratory duplicate for a minimum of 10% of field samples or at least one within each analysis batch. Calculate the relative percent difference (RPD).	RPD must be ±15%.
Section 8.2.2.8.	Laboratory Fortified Blank (LFB)Duplicates	Quarterly, replicates of LFB's are run & included on the on-going charts.	Duplicate Recovery must be 80-120%.
Section 8.1.1.2.	Quality Control Sample	Analyzed Quarterly – from 2nd source	The QCS must be ± 10% of the true value
Section 8.1.2.	MDL Determination	Every six months or whenever a significant change has occurred	

Table 6. Typical Analytical Sequence with Quality Control Requirements

Injection #	Description of Quality Control Standards and Samples	Chapter 2 Acceptance Criteria
	Calibration Blank	
	Level 1 of Initial Calibration	R ≥ 0.9950
	Level 2 of Initial Calibration	Calibration curve to be
	Level 3 of Initial Calibration	done every 6 months
	Level 4 of Initial Calibration	
	Level 5 of Initial Calibration	
1	Instrumentation Blank	
2	QCS (after new calibration curve and quarterly)	90 -110%
3	Initial IPC	90 -110 %
4	LRB	≤ ½ MDL
5	LFB (Duplicates Quarterly)	90 -110 %
6	LLC (Quarterly)	70 -130 %
7	MS (Check Periodically)	80 -120 %
8	Sample 1	
9	Sample 1 – Laboratory Duplicate	
10	Sample 1 – LFM	80-120 %
11- 19	Sample 2 to Sample 10	
20	Continuing IPC	90 -110%
21	Blank	≤ ½ MDL
22	Sample 11	
23	Sample 11 – Laboratory Duplicate	
24	Sample 11 – LFM	
25-34	Sample 12 to Sample 20	
35	Continuing IPC	90 -110%
36	Blank	≤ ½ MDL
37	LFB	90 -110%
38	Sample 21	
39	Sample 21 – Laboratory Duplicate	
40	Sample 21 – LFM	
41	Sample 22…. And so forth	
Last Injections	Ending IPC Calibration Blank LRB	Criteria As Above

Figure 1. Inorganics Primary Standards Logbook

Barnstable County Laboratory

Inorganics Primary Standard Logbook

Date	Analyte	Vendor	Catalog No	Lot No	Concentration	Primary Standard ID	Exp Date	Initial

Figure 2. Inorganics Working Standards Logbook

Barnstable County Laboratory

Inorganics Working Standards Logbook

Date	Analyte	Primary Standard ID	Initial Conc	Amt taken	Final Vol	Final Conc	Solvent	Working Standard ID	Exp Date	Initial

Barnstable County Department of Health and the
Environment Laboratory

EPA Method 350.1

STANDARD OPERATING PROCEDURE

For

Determination of Ammonia Nitrogen in Aqueous Samples
by Semi-Automated Colorimetry

(Revision 001)

January 7, 2015

		Signature	Date
Analyst:	Kelby Karnes		12-2-15
Laboratory Director:	Gongmin Lei		12/2/2015

STANDARD OPERATING PROCEDURE (SOP)

For

Determination of Ammonia Nitrogen in Aqueous Samples
by Semi-Automated Colorimetry

1.0 SCOPE AND APPLICATION

1.1 This SOP covers the determination of ammonia in drinking, ground, and surface waters, domestic and industrial wastes.

1.2 The applicable range is 0.05 – 10 mg/L NH_3 as N. The range may be extended with sample dilution.

2.0 SUMMARY OF METHOD

2.1 The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and distilled into a solution of sulfuric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured colorimetrically.

3.0 DEFINITIONS

3.1 Calibration Blank (CB) – A volume of reagent water fortified with the same matrix as the calibration standards, but without the analyte.

3.2 Calibration Standard (CAL) – A solution prepared from the primary dilution standard or stock standard solutions.

3.3 Instrument Performance Check Solution (IPC) – A Solution of one or more method analytes or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4 Laboratory Fortified Blank (LFB) – An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5   Laboratory Fortified Sample Matrix (LFM) – An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.6   Laboratory Reagent Blank (LRB) – An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7   Linear Calibration Range (LCR) – The concentration range over which the instrument response is linear.

3.8   Safety Data Sheets (SDS) [Used to be called as Material Safety Data Sheet (MSDS)] – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9   Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10   Quality Control Sample (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11   Stock Standard Solution (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.0   INTERFERENCES

4.1   Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the pH of 9.5 at which distillation is carried out.

4.2   Residual chlorine must be removed by pretreatment of the sample with sodium thiosulfate before distillation.

4.3   Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0   SAFETY

5.1   The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical must be regarded as a potential health hazard and exposure must be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2   Barnstable County Health Laboratory maintains a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. Reference files of Safety Data Sheets (SDS) are available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3   The following chemicals have the potential to be highly toxic or hazardous, consult SDS.

5.3.1   Sulfuric acid.

5.3.2   Sodium nitroprusside.

5.3.3   Phenol.

6.0   EQUIPMENT AND SUPPLIES

6.1   Balance – Analytical, capable of accurately weighing to the nearest 0.0001g (Fisher Scientific, Model ACCU-124D).

6.2   Glassware – Class A volumetric flasks and pipets as required.

6.3   SimpleDist Distillation System (Catalog #: C6000; Environmental Express):

6.3.1   12-position system;
6.3.2   12 50 mL boiling tubes;
6.3.3   36-well hot block heating system.

6.4   Automated Continuous Flow Analysis Equipment – QuickChem 8500 Series 2 Flow Injection Analysis System (LACHAT Instruments, A Hach Company Brand)

6.4.1   LACHAT XYZ Autosampler.

6.5 Porous Boiling Chips, VWR Scientific, Inc. Cat No. 26397-409

6.6 Orion 250A+ pH meter,

7.0 REAGENTS, CHEMICALS AND STANDARDS

7.1 Phenol (C_6H_5OH), Fisher, Cat No. 50-146-208, Cat No. A9311-500

7.2 Sulfuric Acid (H_2SO_4), Fisher, Cat No. A300-212

7.3 Sodium Thiosulfate Pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$), Fisher, Cat No. RDC50930-500B1

7.4 Sodium Sulfite Anhydrous (Na_2SO_3), Fisher, Cat No. RDC50870-500B1

7.5 Sodium Tetraborate Decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), Fisher, Cat No. AA4011436

7.6 Sodium Hydroxide (NaOH), Fisher, Cat No. S613-3

7.7 Sodium Hypochlorite (NaClO), Fisher, Cat No. 19-546-929

7.8 Disodium EDTA, (Ethylenediamine Tetraacetic Acid Dihydrate) ($Na_2EDTA \cdot H_2O$), Fisher, Cat No. BP120500

7.9 Sodium Nitroprusside (Sodium Nitroferricyanide Dihydrate) [$Na_2Fe(CN)_5NO \cdot H_2O$], Fisher, Cat No. AC21164-1000

7.10 Ammonium Chloride (NH_4Cl), Fisher, Cat No. AC199975000 and A661-3

7.11 Reagent Water: Ammonia free deionized water produced from Millipore Milli-Q Water Purification System.

7.12 Degassing with Helium:

7.12.1 To prevent bubble formation, degas the carrier and buffer with helium. Use He at 140 kPa (20 lb/in2) through a helium degassing tube. Bubble helium through one liter of solution for one minute.

7.12.2 All reagents used in heated chemistry must be degassed.

7.13 Reagent 1: Sodium Phenolate

7.14.1 Caution: Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin.

7.14.2 In a 1 Liter volumetric flask dissolve 83 g crystalline phenol $[C_6H_5OH]$ in approximately 600 mL reagent water. While stirring slowly add 32 g sodium hydroxide [NaOH]. Cool, dilute to the mark, and invert to mix. DO NOT degas this reagent.

7.15 Reagent 2: Sodium Hypochlorite

In a 500 mL volumetric flask, dilute 250 mL Regular Clorox bleach [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] to the mark with reagent water. Invert to mix.

7.16 Reagent 3: Buffer

In a 1 liter volumetric flask, dissolve 50 g disodium ethylenediamine tetraacetate dihydrate $[Na_2EDTA \cdot 2H_2O]$ and 9.0 g sodium hydroxide [NaOH] in approximately 900 mL reagent water. Dilute to the mark and mix with a magnetic stirrer until dissolved.

7.17 Reagent 4: Sodium Nitroprusside

Dissolve 3.5 g of sodium nitroprusside (CASRN 14402-89-2) into 1 Liter volumetric flask and dilute to the mark with reagent water.

7.18 Reagent 5: Carrier / Diluent and Catch Solution for distillation (0.04N Sulfuric Acid)

To a 1.0 liter volumetric flask, add approximately 900 mL reagent water. Then add 1.099 mL of concentrated sulfuric acid $[H_2SO_4]$. Dilute to the mark with reagent water and invert to mix.

7.19 Borate Buffer Solution, pH=9.5

7.19.1 0.025M Sodium tetraborate - Add 9.5 g of sodium tetraborate decahydrate $[Na_2B_4O_7 \cdot 10H_2O]$ to approximately 500 mL of reagent water in a 1 Liter volumetric flask, mix, and bring to volume with reagent water.

7.19.2 Add 88mL of 0.1N NaOH to 500mL 0.025M $Na_2B_4O_7$ and dilute to 1L with reagent water.

7.20 Sodium Hydroxide solutions

7.20.1 1 N NaOH - Add 40 g of NaOH to 1000 mL of reagent water in a 1 liter volumetric flask, mix and bring to volume.

7.20.2 0.1N NaOH - Dissolve 4.0g NaOH in 1000 mL of reagent water

7.21 Dechlorinating Reagents:

7.21.1 Sodium Thiosulfate: Dissolve 3.5 g of $Na_2S_2O_3 \cdot 5H_2O$ in reagent water and dilute to 1 liter. One mL removes 1 mg/L of residual chlorine in 500 mL of sample.

7.21.2 Sodium Sulfite: Dissolve 0.9 g Na_2SO_3 in reagent water and dilute to 1 liter. One mL removes 1 mg/L residual chlorine per 500 mL of sample.

7.22 Boiling Chips – Rinse boiling chips with sodium hydroxide solution, drain, and remove all traces of sodium hydroxide solution by rinsing several times with reagent water. Air dry overnight and then dry at 180°C for at least 1 hour.

7.23 Calibration Standards

7.23.1 Standard 1 (S1): Stock Standard: 1000 mg/L

In a 1.0 liter volumetric flask, dissolve 3.819 ammonia chloride (NH_4Cl) that has been dried for two hours at 110°C in about 800 mL reagent water. Dilute to the mark with reagent water and invert to mix.

7.23.2 Standard 2 (S2): Intermediate Stock Standard: 20.0 mg N/L in 0.04N H_2SO_4

In a 1 Liter volumetric flask, add 20.0 mL of the stock standard (**Standard 1**) to approximately 900 mL reagent water and then 1.099 mL of concentrated sulfuric acid. Dilute the mark with reagent water, and invert to mix.

7.23..3 Calibration Standards: Using Standard 1 (S1) and 2 (S2) (Section 7.23.1, Section 7.23.2) to prepare the series of standards, as shown below, covering the desired range and a blank by diluting suitable volumes of standard solution with Reagent 5 (Section 7.18).

Initial Calibration Standard (ICS)	Concentration (mg/L)	Volume Taken from S1 or S2 (mL)	Final Volume (mL)
Level 6	10	1.0 (S1)	100
Level 5	5	0.5 (S1)	100
Level 4	0.5	2.5 (S2)	100
Level 3	0.1	0.5 (S2)	100
Level 2	0.05	0.25 (S2)	100
Level 1	0	0	

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples are collected in disposable plastic. Volume collected must be sufficient to insure a representative sample, allow for replicate analysis, and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH<2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

9.0 QUALITY CONTROL

9.1 Barnstable County Health Laboratory operates a formal quality control (QC) program. The QC program for this method consists of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks and other laboratory solutions as a continuing check on performance. The laboratory maintains performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges

and analysis of QCS) and laboratory performance (determination of MDL) prior to performing analyses by this method.

9.2.2 Linear Calibration Range (LCR) – The LCR is determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity uses a blank and five calibration standards. If any verification data exceeds the initial values by ±10%, linearity will be reestablished.

9.2.3 Quality Control Sample (QCS) – The QCS is analyzed right after initial calibration (Section 9.2.2) to verify the calibration standards and acceptable instrument performance with preparation and analysis of a QCS. If the determined concentrations are not within ±10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) – MDL must be established using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, seven replicate aliquots of the fortified reagent water are taken and processed through the entire analytical method. The following equation is used to calculate the MDL:

$$MDL = (t) \times (S)$$

Where

$t =$ Student's value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

$S =$ Standard deviation of the replicate analyses.

9.2.4.1 The Standard deviation (S) can be calculated using the following equation:

$$S = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Where,   n = number of samples;
          x = concentration in each sample.

9.2.4.2 MDLs must be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.2.4.3 One set of MDLs is listed as follows:

Unit: mg/L

Spiking Level	11/10/15	11/10/15	11/10/15	11/13/15	11/13/15	11/19/15	11/19/15	MEAN	STDEV	MDL
0.05	0.039	0.042	0.045	0.032	0.029	0.024	0.026	0.03385	0.008	0.026

9.3   ASSESSING LABORATORY PERFORMANCE

9.3.1   Laboratory Reagent Blank (LRB) – The laboratory analyzes at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination must be suspected and corrective actions must be taken before continuing the analysis.

9.3.2   Laboratory Fortified Blank (LFB) – The laboratory analyzes at least one LFB with each batch of samples. Calculate accuracy as percent recovery as follows:

$$R = \frac{C_s - C}{S} \times 100$$

Where,   R =   percent recovery;
Cs =   recovered fortified blank concentration;
C =   blank background concentration;
S =   concentration equivalent of analyte added to blank.

9.3.2.1 If the recovery of any analyte falls outside the required control limits of 90-110%, the result is judged out of control, and the source of the problem must be identified and resolved before continuing analysis.

9.3.3   The laboratory also uses LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When

sufficient internal performance data become available (usually a minimum of 25 analyses), optional control limits and control charts can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x – 3S

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also the standard deviation (S) data must be used to establish an on-going precision statement for the level of concentration included in the LFB. These data are kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) – For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every 10th sample (or more frequently, if required), and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±10%. If the calibration cannot be verified within the specified limits, the IPC solution is reanalyzed. If the second analysis of the IPC solution confirms calibration to be outside the limits, the sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution are kept on file with the sample analysis data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) – The laboratory adds a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2 The percent recovery for ammonia is calculated and corrected for concentration measured in the unfortified sample using the following equation:

$$R = \frac{C_s - C}{S} \times 100$$

Where, $R =$ percent recovery;
$Cs =$ fortified sample concentration;
$C =$ sample background concentration;
$S =$ concentration equivalent of analyte added to sample.

Acceptable range of R is 90-110%.

9.4.3 If the recovery falls outside the designated LFM recovery range (90-110%) and the laboratory performance is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be matrix related, not system related.

10   CALIBRATION AND STANDARDIZATION

10.1   Prepare reagent and standards as described in Section 7.

10.2   Set up the Ammonia manifold as shown in Section 17.3 (Ammonia) of the Lachat Instruments Methods Manual (Section 14.2)

10.3   Input data system parameters as shown in Section 17 (Ammonia) of the Lachat Instruments Methods Manual (Section 14.2)

10.4   Pump reagent water through all reagent lines and check for leaks and smooth flow. In order to avoid precipitate forming in the manifold tubing: Add the **Buffer Line First** and allow to pump through manifold for at least 5 minutes. Then the Carrier and other reagent lines one by one, ending with the nitroprusside added last. For removal after analysis, reverse this order with the nitroprusside line disconnected first, and the buffer line last. When finished, place all respective reagent lines into water and allow to pump through manifold for ten minutes.

10.5   Place standards in the sampler and sequence the required information in the data system.

10.6 Calibrate the instrument by injecting the standards. The system will then associate the concentrations with the peak area for each standard to determine the calibration curve.

10.7 The initial calibration is deemed acceptable if the following criteria are met:

10.7.1 $R \geq 0.995$

10.7.2 Quality Control Sample (QCS) standard is run right after the initial calibration. The concentration of the QCS is 10 mg/L. This standard (Ammonium chloride) is ordered from Fisher Scientific, Acros Organics, ACS reagent grade. The procedure for making the QCS is the same as the one for ICS 2 of the calibration standards described in (Section 7.23.1, 7.23.2, and 7.23.3.) The QCS concentration must fall within ± 10% of the stated value.

10.7.3 Instrument Performance Check (IPC) refer to (Section 9.3.4).

10.8 Figure 1. Lists a set of initial calibration peaks and a linear calibration curve

11 PROCEDURE

11.1 DISTILLATION

11.1.1 Adjust an aliquot of 25mL of sample to pH of 9.5 using either 0.1N NaOH or 1N NaOH and remove any residual chlorine.

11.1.2 Pipette 25mL of pH-adjusted sample or an aliquot diluted to 25mL with reagent water into the SimpleDist System boiling tube. Add boiling chips to the boiling tube.

11.1.3 Insert the inlet part liner into the green screw cap and thread assembly onto the top of the boiling tube.

11.1.4 Assemble the reagent addition tube with funnel tip and insert into the shorter port on the cap.

11.1.5 Pipette 20mL of 0.04N Catch Solution (Reagent 5) into the collection trap. Attach the collection trap to the boiling tube and attach the tubing/adaptor from the vacuum manifold to the collection trap. Repeat for all samples.

11.1.6 Turn on the vacuum and adjust each valve to provide an air flow bubble rate of 10-15 bubbles per second for each position as viewed in the collection trap.

11.1.7 The vacuum should be sufficient to maintain slight negative pressure on the assembly throughout the distillation.

11.1.8 Monitor the vacuum to insure a back pressure does not build up in the boiling tube.

11.1.9 Add 1.25 mL of borate buffer to the sample through the reagent inlet tube.

11.1.10 Turn on the hot block and set the temperature to 135°C.

11.1.11 Heat the samples for 60minutes after the hot block reaches the 135°C temperature.

11.1.12 Without turning off the vacuum, remove the collection trap.

11.1.13 Turn the air / vacuum valves off.

11.1.14 Bring the volume of the distillate in the collection trap up to 25 mL with the 0.04N sulfuric acid catch solution.

11.1.15 The distillate in the collection trap is now ready for analysis.

11.1.16 Position the vent tube of the collection trap up to prevent the trapping of air and pour the contents of the trap into an appropriate container.

11.1.17 Clean exterior surfaces with a damp sponge. For acid spills, sponge surfaces with a diluted solution of sodium carbonate followed by distilled water.

11.2 FLOW INJECTION SYSTEM START-UP PROCEDURE

11.2.1 Prepare reagent and standards as described in section 7.

11.2.2 Set up manifold as shown in Section 17.2 of the Lachat Instruments Methods Manual.

11.2.3 Input peak timing and integration window parameters as specified in section 17.2 of the Lachat Instruments Methods Manual.

11.2.4 Pump reagent water through all the reagent lines and check for leaks and smooth flow. Switch to reagent lines - add buffer first and pump through the system for 5 minutes, followed by the other reagents, adding salicylate nitroprusside last – and allow the system to equilibrate until a stable baseline is achieved.

11.2.5 Place the standards in the autosampler, and fill the sample tray. Input the information required by the data system, such as concentration, replicates and QC scheme.

11.2.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.2.7 After a stable baseline has been obtained, start the sampler and perform the analysis.

11.3 ANALYTICAL SEQUENCE

Please see Table 1 for analytical sequence.

11.4 TROUBLESHOOTING AND SYSTEM NOTES

11.4.1 Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2 If sample concentrations are greater than the high standard the distilled sample should be diluted with **Reagent 5 (Carrier.)** Do not dilute distilled samples or standards with reagent water, as this will cause a problem with matix-matching, pH differences.

11.4.3 If the distillation tubes are not completely dry and have water droplets on them, there exists the possibility of ammonia contamination in the water droplets.

11.4.4 If phosphorus is also determined with the Lachat System, a second helium degassing tube should be used and segregated for the individual chemistries.

11.4.5 If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

11.4.5.1 Place transmission lines in water and pump to clear reagents first.

11.4.5.2 Place reagent lines in 1M HCl and pump for several minutes

11.4.5.3 Place all lines back into water and pump out HCl.

12 POLLUTION PREVENTION

12.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in the laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice.

12.2 Quantity of the chemicals purchased should be based on the expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

13 WASTE MANAGEMENT

13.1 The laboratory waste management practices are conducted consistent with all applicable rules and regulations as stated in the laboratory's Sample and Waste Disposal (Revision 001) on February 25, 2004. Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

14 REFERENCES

14.1 U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1993, Method 350.1

14.2 Lachat Instruments Methods Manual, QuikChem Method 10-107-06-1-B, Revision Date, 27 August 2001.

Figure 1.

Figure : 1 (Ammonia)

Area = 9.205 * Conc + 0.207
Conc = 0.109 * Area - 0.022
Correlation Coefficient (r) = 0.99998
Weighting : 1/x
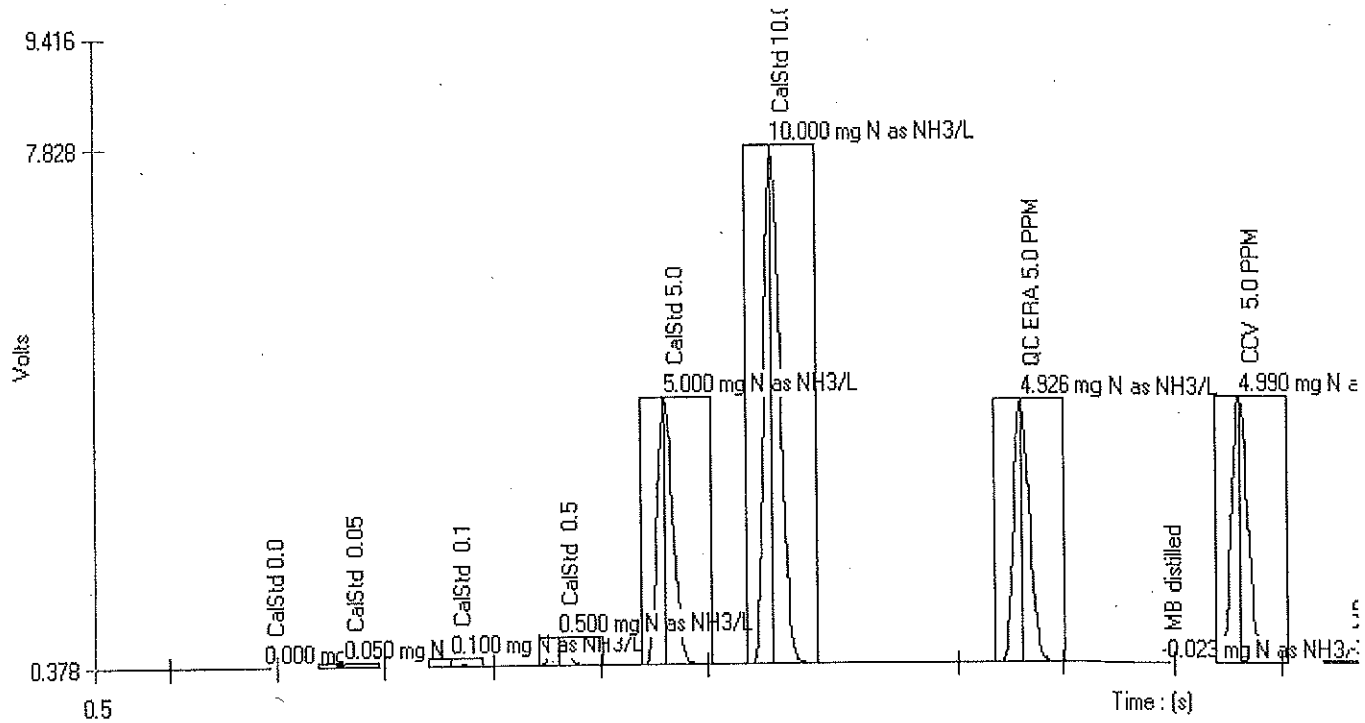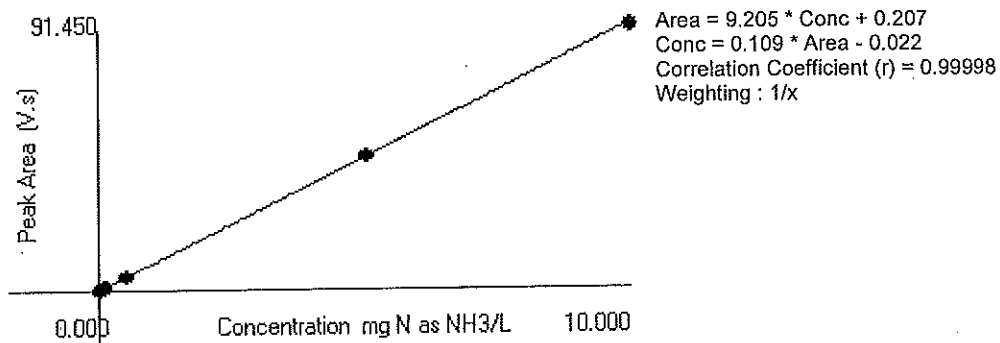
Table 1.   A Typical Analytical Sequence with Quality Control Requirements

Injection #	Description of Quality Control Standards and Samples	1. Acceptance Criteria
1	Level 1 (0 mg/L) of Initial Calibration	R ≥ 0.995
2	Level 2 (0.05 mg/L) of Initial Calibration	
3	Level 3 (0.1 mg/L) of Initial Calibration	
4	Level 4 (0.5 mg/L) of Initial Calibration	
5	Level 5 (5.0 mg/L) of Initial Calibration	
6	Level 6 (10 mg/L) of Initial Calibration	
7	QCS at 5.0 mg/L	90-110%
8	Blank	
9	CCV at 5.0 mg/L	90-110%
10	MB	
11	LFB at 5.0 mg/L	90-110%
12	Sample 1	
13	Sample 1 – Laboratory Duplicate	
14	Sample 1 - Matrix Spike	
15	Sample 2	
16	Sample 3	
17	Sample 4	
18	Sample 5	
19	Sample 6	
20	Sample 7	
21	Sample 8	
22	Sample 9	
23	Sample 10	
24	Blank	
25	CCV	90-110%
26	MB	
27-35	Sample 11 to Sample 19	
36	Sample 20	
37	Blank	
38	CCV at 5.0 mg/L	90-110%
39	MB	
40	LFB at 5.0 mg/L	90-110%

Barnstable County Health Laboratory

September 29, 2015

Barnstable County Department of Health and the
Environment Laboratory

EPA Method 351.2

STANDARD OPERATING PROCEDURE

For

Determination of Total Kjeldahl Nitrogen in Aqueous Samples
by Semi-Automated Colorimetry

(Revision 001)

September 29, 2015

		Signature	Date
Analyst:	Kelby Karnes	_____	
Laboratory Director:	Gongmin Lei	_____	

September 29, 2015

STANDARD OPERATING PROCEDURE (SOP)

For

Determination of Total Kjeldahl Nitrogen in Aqueous Samples
by Semi-Automated Colorimetry

1.0   SCOPE AND APPLICATION

1.1   This SOP provides procedure for determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

1.2   The applicable range is 0.1 – 20 mg/L TKN. The range may be extended with sample dilution.

2.0   SUMMARY OF METHOD

2.1   The sample is heated in the presence of sulfuric acid, H_2SO_4 for three hours. The residue is cooled, diluted to 25 mL and analyzed for ammonia. The digested sample may also be used for phosphorus determination.

2.2   Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described.

2.3   Organic Kjeldahl nitrogen is the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value.

3.0   DEFINITIONS

3.1   Calibration Blank (CB) – A volume of reagent water fortified with the same matrix as the calibration standards, but without the analyte .

3.2   Calibration Standard (CAL) – A solution prepared from the primary dilution standard or stock standard solutions.

3.3   Instrument Performance Check Solution (IPC) – A Solution of one or more method analytes or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4   Laboratory Fortified Blank (LFB) – An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5   Laboratory Fortified Sample Matrix (LFM) – An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.6   Laboratory Reagent Blank (LRB) – An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7   Linear Calibration Range (LCR) – The concentration range over which the instrument response is linear.

3.8   Safety Data Sheets (SDS) [Used to be called as Material Safety Data Sheet (MSDS)] – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9   Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10   Quality Control Sample (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11   Stock Standard Solution (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.0   **INTERFERENCES**

4.1   High nitrate concentrations (10x or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

4.2   Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0   SAFETY

5.1   The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical must be regarded as a potential health hazard and exposure must be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2   Barnstable County Health Laboratory maintains a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. Reference files of Safety Data Sheets (SDS) are available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3   The following chemicals have the potential to be highly toxic or hazardous, consult SDS.

5.3.1   Sulfuric acid.

5.3.2   Sodium nitroprusside.

6.0   EQUIPMENT AND SUPPLIES

6.1   Balance – Analytical, capable of accurately weighing to the nearest 0.0001g. Fisher Scientific, Model ACCU-124D Dual Range.

6.2   Glassware – Class A volumetric flasks and pipets as required.

6.3   Block Digestor with Tubes – TKN 50 well AIM600 Block/Controller with rack and 100 mL glass digestion tubes (Environmental Express, Item#: SC900).

6.4   Automated Continuous Flow Analysis Equipment – QuickChem 8500 Series 2 Flow Injection Analysis System (LACHAT Instruments, A Hach Company Brand)

6.4.1   LACHAT XYZ Autosampler.

6.5   BD Kjeldahl Digestion Granules from Environmental Express, Item#. 8032178

6.6   Seal Analytical Teardrop Stoppers, Item No. SC9703

7.0   **REAGENTS, CHEMICALS AND STANDARDS**

7.1   Potassium Sulfate (K_2SO_4). Fisher, Cat No. P305-500

7.2   Copper (II) Sulfate ($CuSO_4$). Fisher, Cat No. AC422871000

7.3   Sulfuric Acid (H_2SO_4). Fisher, Cat No. A300-212

7.4   Sodium Hypochlorite (NaClO). Cat No. 19-546-929

7.5   Sodium Salicylate $C_6H_4(OH)(COO)Na$. Fisher, Cat No. 50-700-6201

7.6   Sodium Nitroprusside [sodium nitroferricyanide dehydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$]. Fisher, Cat No. AC21164-1000

7.7   Sodium Phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$). Fisher, Cat No. AC20651-5000

7.8   disodium EDTA (ethylenediaminetetracetic acid salt). Fisher, Cat No. BP120500

7.9   Sodium Hydroxide (NaOH). Fisher, Cat No. S613-3

7.10   Ammonium Chloride (NH_4Cl). Fisher, Cat No.^{s} AC199975000 and A661-3

7.11   **Reagent Water**:   Ammonia free deionized water produced from Millipore Milli-Q Water Purification System.

7.12   **Degassing with Helium**:

   7.2.1   To prevent bubble formation, degas the carrier and buffer with helium. Use He at 140 kPa (20 lb/in2) through a helium degassing tube. Bubble helium through one liter of solution for one minute.

   7.2.2   All reagents used in heated chemistry must be degassed.

7.13   **Reagent 1:   Digestion Solution**

   In a 1.0 liter volumetric flask, add 134 g potassium sulfate (K_2SO_4) and 7.3 g copper sulfate ($CuSO_4$) in 800 mL water. Then add 134 mL conc.

Sulfuric acid (H_2SO_4) and dilute to the mark with reagent water. Stir to mix.

7.14   Reagent 2:   Hypochlorite Solution

In a 250 mL volumetric flask, dilute 15 mL 5.25% sodium hypochlorite (NaOCl) to the mark with reagent water. Invert to mix.

7.15   Reagent 3:   Salicylate Nitroprusside

In a 1.0 liter volumetric flask, dissolve 150 g sodium salicylate [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$] and 1.0 g sodium nitroprusside [sodium nitroferricyanide dehydrate, $Na_2Fe(CN)_5NO·2H_2O$] in about 800 mL reagent water. Dilute to the mark with reagent water and invert to mix. Store in a dark bottle and prepare fresh monthly.

7.16   Reagent 4:   Buffer

In a 1 liter volumetric flask containing 900 mL reagent water, completely dissolve 35 g sodium phosphate dibasic heptahydrate ($Na_2HPO_4·7H_2O$). Next, add 20 g disodium EDTA (ethylenediaminetetracetic acid salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 50 g sodium hydroxide (NaOH), dilute to the mark with reagent water and invert to mix. Degas weekly and prepare fresh monthly.

7.17   Reagent 5:   Sodium Hydroxide (0.8M)

In a 1.0 liter volumetric flask, dissolve 32 g sodium hydroxide (NaOH) in about 800 mL reagent water. Dilute to the mark with reagent water and stir to mix.

7.18   Reagent 6:   Digestion Diluent (for Carrier and Simulated Standards)

In a 1.0 liter volumetric flask, dissolve 400 mL digestion solution (**Reagent 1**) in about 600 mL reagent water. Dilute to the mark with reagent water and shake to mix.

7.19   Calibration Standards

7.19.1 Stock Standard: 1000 mg/L

In a 1.0 liter volumetric flask, dissolve 3.819 ammonium chloride (NH_4Cl) that has been dried for two hours at 110˚C in about 800 mL reagent water. Dilute to the mark with reagent water and invert to mix.

7.19.2 Working Stock Standard: 20 mg N/L

In a 250 mL volumetric flask, dilute 5.0 mL of the stock standard (1000 mg/L) to the mark with reagent water or digestion diluent: Depending on which process utilized: **Digested** (use reagent water) for Samples, LFB, Blanks, and **Simulated Digestion** (use digestion diluent) for Calibration Standards and QCS, and invert to mix.

7.19.3 Calibration Standards: **For Simulated Digestion**

There are six levels calibration standards and their respective concentrations and preparation procedures are listed as follows:

Level	Concentration (mg N/L)	Volume (mL) of Working Stock Standard (20 mg N/L) to 50 mL with Digestion Diluent
6	20	50
5	10	25
4	5.0	12.5
3	1.0	2.5
2	0.50	1.25
1	0	0

8.0   SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1   Samples are collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected must be sufficient to insure a representative sample, allow for replicate analysis, and minimize waste disposal.

8.2   Samples must be preserved with H_2SO_4 to a pH<2 and cooled to 4˚C at the time of collection.

8.3   Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4˚C and may be held for up to 28 days.

9.0   QUALITY CONTROL

9.1   Barnstable County Health Laboratory operates a formal quality control (QC) program. The QC program for this method consists of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks and other laboratory solutions as a continuing

check on performance. The laboratory maintains performance records that define the quality of the data that are generated.

9.2   INITIAL DEMONSTRATION OF PERFORMANCE

    9.2.1   The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of QCS) and laboratory performance (determination of MDL) prior to performing analyses by this method.

    9.2.2   Linear Calibration Range (LCR) – The LCR is determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity uses a blank and five calibration standards. If any verification data exceeds the initial values by ±10%, linearity will be reestablished.

    9.2.3   Quality Control Sample (QCS) – The QCS is analyzed right after initial calibration (Section 9.2.2) to verify the calibration standards and acceptable instrument performance with preparation and analysis of a QCS. If the determined concentrations are not within ±10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

    9.2.4   Method Detection Limit (MDL) – MDL must be established using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, seven replicate aliquots of the fortified reagent water are taken and processed through the entire analytical method. The following equation is used to calculate the MDL:

$$MDL = (t) \times (S)$$

Where

        $t =$ Student's value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

        $S =$ Standard deviation of the replicate analyses.

    9.2.4.1 The Standard deviation (S) can be calculated using the following equation:

$$S = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Where,   n = number of samples;
             x = concentration in each sample.

9.2.4.2 MDLs must be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.2.4.3 One set of MDLs is listed as follows:

									Unit: mg/L
Spiking Level	**8/11/15**	**8/11/15**	**8/11/15**	**8/31/15**	**8/31/15**	**8/31/15**	**9/1/15**	**MEAN**	**STDEV**	**MDL**
0.5	0.391	0.419	0.346	0.268	0.344	0.362	0.399	0.361	0.050	0.16

9.3   ASSESSING LABORATORY PERFORMANCE

9.3.1   Laboratory Reagent Blank (LRB) – The laboratory analyzes at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination must be suspected and corrective actions must be taken before continuing the analysis.

9.3.2   Laboratory Fortified Blank (LFB) – The laboratory analyzes at least one LFB with each batch of samples. Calculate accuracy as percent recovery as follows:

$$R = \frac{C_s - C}{S} \times 100$$

Where,   R =   percent recovery;
             Cs =   recovered fortified blank concentration;
             C =   blank background concentration;
             S =   concentration equivalent of analyte added to blank.

9.3.2.1 If the recovery of any analyte falls outside the required control limits of 90-110%, the result is judged out of control, and the source of the problem must be identified and resolved before continuing analysis.

9.3.3 The laboratory also uses LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 25 analyses), optional control limits and control charts can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x − 3S

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also the standard deviation (S) data must be used to establish an on-going precision statement for the level of concentration included in the LFB. These data are kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) – For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every 10th sample (or more frequently, if required), and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±10%. If the calibration cannot be verified within the specified limits, the IPC solution is reanalyzed. If the second analysis of the IPC solution confirms calibration to be outside the limits, the sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution are kept on file with the sample analysis data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) – The laboratory adds a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte

concentration should be the same as that used in the laboratory fortified blank.

9.4.2 The percent recovery for TKN is calculated and corrected for concentration measured in the unfortified sample using the following equation:

$$R = \frac{C_s - C}{S} \times 100$$

Where, R = percent recovery;
Cs = fortified sample concentration;
C = sample background concentration;
S = concentration equivalent of analyte added to sample.

Acceptable range of R is 90-110%.

9.4.3 If the recovery falls outside the designated LFM recovery range (90-110%) and the laboratory performance is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be matrix related, not system related.

10 CALIBRATION AND STANDARDIZATION

10.1 Prepare reagents and standards as described in Section 7.

10.2 Set up TKN manifold as is shown in Section 17.3 of the Lachat Instruments Methods Manual (reference 14.5.)

10.3 Input data system parameters as shown in Section 17 of the Lachat Instruments Methods Manual (reference 14.5.)

10.4 Pump reagent water through all reagent lines and check for leaks and smooth flow. In order to avoid precipitate forming in the manifold tubing: Add the **Buffer Line First** and allow to pump through manifold for at least 5 minutes. Then add reagent lines one by one, ending with the salicylate nitroprusside added last. For removal after analysis, reverse this order with the salicylate nitroprusside line disconnected first, and the buffer line last. When finished, place all respective reagent lines into water and allow to pump through manifold for ten minutes.

10.5 Place standards in the sampler and sequence the required information in the data system.

10.6 Calibrate the instrument by injecting the standards. The system will then associate the concentrations with the peak area for each standard to determine the calibration curve.

10.7 The initial calibration is deemed acceptable if the following criteria are met:

10.7.1 R $\geq$ 0.995

10.7.2 Quality Control Sample (QCS) standard is run right after the initial calibration. The concentration of the QCS is 10 mg/L. This standard (Ammonium chloride) is ordered from Fisher Scientific, Acros Organics, ACS reagent grade. The procedure for making the QCS is the same as the one for Level 5 of the calibration standards described in (Section 7.9.1, 7.9.2, and 7.9.3). The QCS concentration must fall within $\pm$ 10% of the stated value.

10.7.3 Instrument Performance Check (IPC) refer to (Section 9.3.4).

10.8 **Figure 1.** Lists a set of initial calibration peaks and a linear calibration curve

11 PROCEDURE

11.1 Barnstable County Health Laboratory prepares the standards using the simulated digestion process with the digestion diluent. At a minimum, two blanks and one standard (LFB) should be prepared in reagent water and carried through the digestion procedure.

11.2 **DIGESTION PROCEDURE**

11.2.1 To a **25.0 mL** sample add **10 mL digestion solution** (Reagent 1) and mix.

11.2.2 Add 2 to 4 BD Kjeldahl Digestion Granules to each tube.

11.2.3 Place tubes in the preheated block digester for one hour at 200°C. Water from the sample must be boiled off before increasing the temperature.

11.2.4 Place the cold finger, teardrop stopper on the top of the sample tube.

11.2.5 Continue to digest for 2 hours at 380°C. This includes the ramp time (approximately 50 minutes) for the block temperature to come up to 380°C.

11.2.6 Remove the sample tubes from the block and allow about 3 minutes to cool.

11.2.7 Dilute to **25.0 mL** with **reagent water (add 23.5 mL)** to each tube and vortex to mix.

11.2.8 If the samples are not run immediately they should be covered tightly and refrigerated at 4°C.

11.3 SYSTEM START-UP PROCEDURE

11.3.1 Prepare reagent and standards as described in section 7.

11.3.2 Set up manifold as shown in Section 17.3 of the Lachat Instruments Methods Manual.

11.3.3 Input peak timing and integration window parameters as specified in section 17.2 of the Lachat Instruments Methods Manual.

11.3.4 Pump reagent water through all the reagent lines and check for leaks and smooth flow. Switch to reagent lines - add buffer first and pump through the system for 5 minutes, followed by the other reagents, adding salicylate nitroprusside last – and allow the system to equilibrate until a stable baseline is achieved.

11.3.5 Place the standards in the autosampler, and fill the sample tray. Input the information required by the data system, such as concentration, replicates and QC scheme.

11.3.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.3.7 After a stable baseline has been obtained, start the sampler and perform the analysis.

11.4 TROUBLESHOOTING AND SYSTEM NOTES

11.4.1 Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2 If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6 (diluent.)** Do

not dilute digested samples or standards with reagent water, as this will cause a problem with matix-matching, pH differences.

11.4.3 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs NaOH can be run through the system to attempt to clear clogs, flush system with NaOH for 20 minutes. If clogged tubing cannot be cleared, the tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer solution.

11.4.4 In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.

11.4.5 If the block digestor tubes are not completely dry and have water droplets on them, there exists the possibility of ammonia contamination in the water droplets.

11.4.6 If phosphorus is also determined with the Lachat System, a second helium degassing tube should be used and segregated for the individual chemistries.

11.4.7 If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

    11.4.7.1 Place transmission lines in water and pump to clear reagents first.

    11.4.7.2 Place reagent lines in 1M HCl and pump for several minutes

    11.4.7.3 Place all lines back into water and pump out HCl.

11.4.8 If digested samples contain turbidity allow to settle prior to analysis, decant sample slowly into test tube.

11.4.9 Alternatively, if turbid conditions persist, filter the digested sample with 0.45uM filter.

12 POLLUTION PREVENTION

12.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in the laboratory operation.

The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice.

12.2 Quantity of the chemicals purchased should be based on the expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
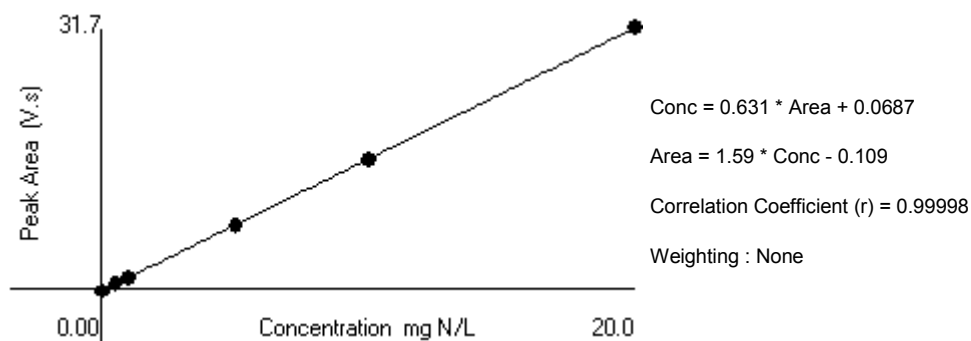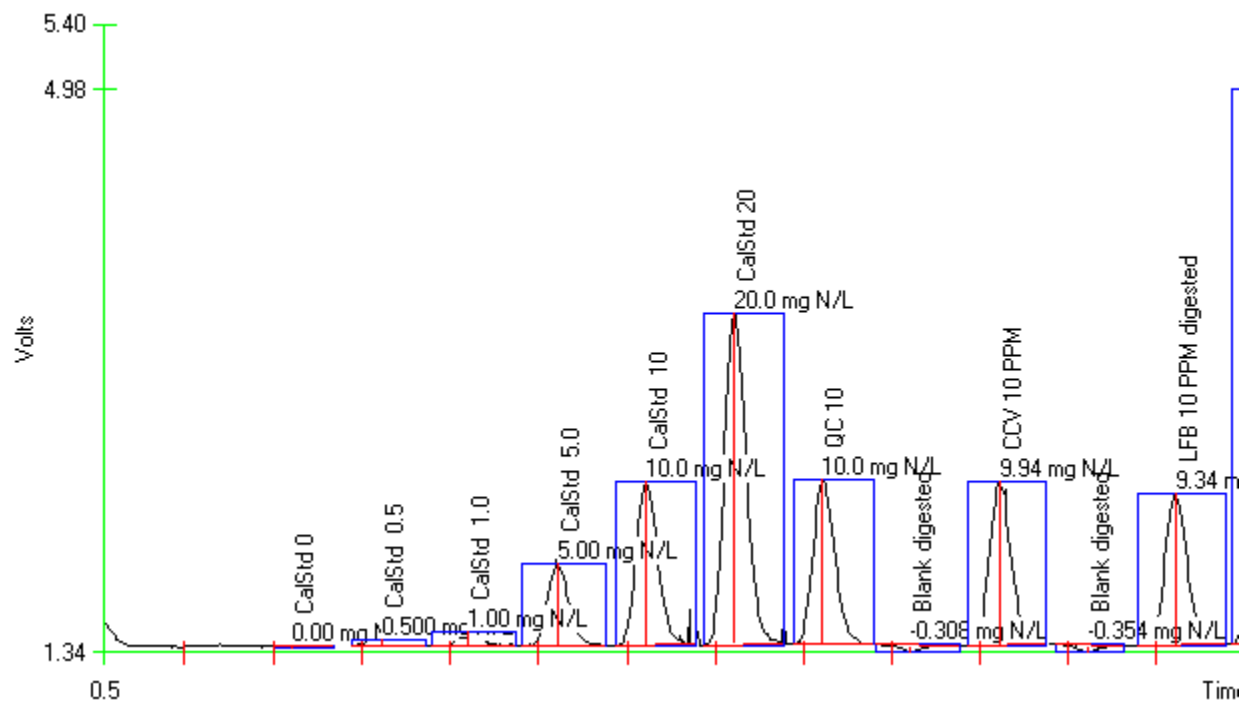
13 WASTE MANAGEMENT

13.1 The laboratory waste management practices are conducted consistent with all applicable rules and regulations as stated in the laboratory's Sample and Waste Disposal (Revision 001) on February 25, 2004. Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

14 REFERENCES

14.1 U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1993, Method 351.2

14.2 ASTM, Water(I), Volume 11.01, Method D3590-89, Test Methods for Kjeldahl Nitrogen in Water, p. 447

14.3 Code of Federal Regulation 40, Chapter 1, Part 136, Appendix B

14.4 Guidelines and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.

14.5 Lachat Instruments Methods Manual, QuikChem Method 10-107-06-2-L, Revision Date, 14 May 2008

Figure 1.

Conc = 0.631 * Area + 0.0687

Area = 1.59 * Conc - 0.109

Correlation Coefficient (r) = 0.99998

Weighting : None

Barnstable County Department of Health and the Environment Laboratory

SM 2540 D

STANDARD OPERATING PROCEDURE

For

Determination of Total Suspended Solids in Aqueous Samples

(Revision 005)

November 16, 2015

		Signature	Date
Analyst:	Diane Brown	_____	
Laboratory Director:	Gongmin Lei	_____	

Barnstable County Laboratory

STANDARD OPERATING PROCEDURE (SOP)
For

Determination of Total Suspended Solids in Aqueous Samples

1. SCOPE AND APPLICATION

1.1 This method covers the determination of total suspended solids (TSS) in drinking water, surface water, domestic water, industrial wastes, and other aqueous samples.

2. SUMMARY OF METHOD

2.1 A well-mixed sample is filtered through a Preweighed glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105 °C.

3. INTERFERNCES

3.1 Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specific because these variables have been shown to affect results.

3.2 Samples high in dissolved solids, such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken to thoroughly wash the filter to ensure removal of dissolved material. Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter.

4. SAFETY

4.1 Follow general laboratory safety guidelines, such as wearing safety glasses, gloves and a lab coat.

5. EQUIPMENT AND SUPPLIES

5.1 ProWeigh Filters, 47mm (Environmental Express, catalog # F93447MM)

5.2 Filtration apparatus with reservoir and a coarse (40-60 microns) fritted disc as a Filter support (VWR, catalog # 28143-550)

5.3 Suction flask, 5 gallons

5.4 Drying Oven (Fisher Scientific Isotemp Oven, Model # 625G, catalog # 13-247-625G) for operation a 103-105 °C.

5.5 Desiccator, provided with a desiccant containing a color indicator of moisture concentration.

5.6 Aluminum weighing dishes (VWR, Catalog # 25433-008)

5.7 Analytical balance (± 0.1mg)- Fisher Scientific (Model ACCU-124D)

5.8 Graduated cylinder, 100mL (Fisher Scientific, catalog # 08572D)

6. REAGENTS AND STANDARDS

6.1 Reagent water – Deionized water from Milli-Q Direct 8/16 System, Millipore Direct-Q 3 System.

6.2 30 mg/L TSS standard from NSI Solutions Catalog # QCI-057 for low level standard check.

7. SAMPLE COLLECTION, PRESERVATION AND STORAGE

7.1 Samples are collected in 1 liter plastic bottles. All bottles must be thoroughly cleaned and rinsed with reagent water.

7.2 Preservation of the sample is not practical; analysis should begin as soon as possible. Samples are shipped iced or stored cold in a refrigerator at 4°C.

7.3 The holding time is 7 days and no preservation is required.

7.4 Non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.

8.   QUALITY CONTROL

8.1   A method blank using 100 mL of deionized is processed with the same procedures as the samples in each batch.

8.2   A duplicate sample is to be included in any set of 10 samples. The agreement between the sample and the duplicate must be < 20%.

8.3   Method Detection Limit (MDL)- An MDL is established using reagent water fortified at a concentration of three to five times the estimated detection limit. seven replicate aliquots of the fortified reagent water are run and processed through the entire analytical method. Then the MDL is calculated as follows:

$$\text{MDL} = (t) \times (S_{n-1}) \tag{1}$$

Where,
 t = student's value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.
 S_{n-1} = sample standard deviation (n-1) of the seven replicate analyses.

 The laboratory conducts MDL study annually. One set of MDL study is shown in Table 1.

8.4   Accuracy and Precision- 4 replicates of a known value are prepared and analyzed. The mean measured concentration ($C_{\bar{x}}$) of the replicate values is calculated as follows:

$$C_{\bar{x}} = \frac{(C_1 + C_2 + C_3 + C_4)}{n}$$

Where,

 $C_{\bar{x}}$ = Mean recovered concentration of the replicate analysis.
 $C_1, C_2, \ldots C_n$= Recovered concentrations of the replicate 1,2, …n.
 The value derived for $C_{\bar{x}}$ must be within $\pm$ 10% of the true value.

 The percent relative standard deviation (%RSD) of the replicate analysis as stated above is calculated using the following equation.

$$\% \text{ RSD} = \frac{(S_{n-1}) x 100}{(C_{\bar{x}})}$$

Where,
 S_{n-1} = sample standard deviation (n-1) of the replicate analysis.
 $C_{\bar{x}}$ = mean recovered concentration of the replicate analysis.

8.5   A low level standard is to be run on an ongoing basis. Use NSI Solutions Catalog

Number QCI-057.

9.   CALIBRATION OF BALANCE

   9.1   Check the analytical balance with three Class 's' weights: 0.05(g), 10(g), and
         100(g) and record them in the Analytical Balance Calibration Logbook.

10.   PROCEDURE

   10.1   Preparation of glass-fiber filter disk:

       10.1. 1 Take a Preweighed 47mm glass fiber filter from Environmental Express
                and record the weight in the Initial Weight column in the Solids Runlog
                Book. Follow this procedure for all samples.

   10.2   Sample Analysis:

       10.2. 1 Assemble filtering apparatus and filter and begin suction. The filter is
                seated on fritted support by wetting it with deionized water. After shake
                thoroughly, the sample volume is measured in a graduated cylinder and
                slowly poured on the filter. The filter is then rinsed with three successive
                10 mL volumes of deionized water, allowing complete drainage between
                washings and suction is continued for three minutes after filtration is
                complete.

       10.2 2   Filters are carefully removed from filtration apparatus and transferred to an
                aluminum weighing dish for support. Filters placed in their aluminum
                weighing dish are put in the oven at 103-105 ° Celsius for at one hour.
                Cool in desiccator to balance temperature and weigh. Repeat the cycle of
                drying , cooling, desiccating and weighing until a constant weight is
                obtained or until weight change is less than 4% of the previous weighing
                or 0.5mg, whichever is less. Store in desiccator until needed.

11.   DATA ANALYSIS, CALCULATIONS AND REPORTS

   11.1   Calculation:

$$\text{TSS (mg/L)} = \frac{(A-B) \times 1000}{V}$$

       Where:
           A= weight of filter + dried residue (mg);
           B= weight of filter (mg);
           V= sample volume (mL).

   11.2   Reports:

All data, including the date, Lab ID, Client ID, sample volume (V), weight of filter (B), weight of filter + dried residue (A), the calculation, and analyst initials are recorded in the Inorganics Solid Runlog Book (Figure 1)

12.   POLLUTION PREVENTION AND WASTE MANAGEMENT

12.1   The laboratory waste management practices are conducted consistent with all Applicable rules and regulations as stated in the laboratory's Sample and Waste Disposal (Revision 003) on July 6,2006. Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

13.   REFERENCES

1.   U.S. Environmental Protection Agency, "Method or Guidance for Analysis of Water, Residue, Non-Filterable (Gravimetric, Dried at 103-105°C)", Method 160.2, Issued 1971.

2.   American Public Health Association (APHA)
American Water Works Association (AWWA)
Water Environment Federation (WGI)
Standard Methods (SM) for the Examination of Water and Wastewater, 22nd edition SM2540D, 2012.

Table 1

Method Detection Limit (MDL) for Total Suspended Solids (TSS) Using SM 2540D								
Unit: mg/L			Spiking Level: 15 mg/L			Analyst: Diane Brown		
	Filter 1	Filter 2	Filter 3	Filter 4	Filter 5	Filter 6	Filter 7	STDEV	MDL
Date Analyzed	1/8/2010	1/8/2010	1/8/2010	1/11/2010	1/11/2010	1/19/2010	1/19/2010		
Concentration	13	14	16	14	15	14	15	0.9759	3.06

Figure 1:

Inorganics Solid Runlog Book

Date	Lab ID	Client ID	Sample Vol (mL)	Initial Wt (g)	Final Wt (g)			TSS (mg/L)	VSS (mg/L)	TS (mg/L)	TDS (mg/L)	Comment	Initial
					Wt01	Wt02	Wt03						

APPENDIX E

Barnstable County Department of Health and Environment Laboratory

STANDARD OPERATING PROCEDURE

For the Determination of
Biochemical Oxygen Demand (BOD$_5$)
and
Carbonaceous Biochemical Oxygen Demand (CBOD$_5$)
in Aqueous Samples Using
SM 5210 B

Revision 010

July, 2015

	Signature	Date
Analysts: Lacey Prior	*Lacey Adams Prior*	12/23/15
Diane Brown	*Diane Brown*	12/23/15
Laboratory Director: Gongmin Lei	*Gongmin Lei*	12/28/2015

Standard Operation Procedure for the Determination of Biochemical Oxygen Demand (BOD$_5$)
and Carbonaceous Biochemical Oxygen Demand (CBOD$_5$)
in Aqueous Samples using SM5210B

1.0   SCOPE AND APPLICATION

The Biochemical Oxygen Demand (BOD) determination is an empirical test used to determine the relative oxygen requirements of wastewater, effluents, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of the treatment system.

2.0 SUMMARY OF METHOD

The test measures the molecular oxygen utilized during a 5-day incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron by the use of microorganisms. It may also measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The method consists of filling an airtight bottle to slightly overflowing with sample & prepared dilution water and incubating it at 20°C for 5 days. Its dissolved oxygen is measured initially and after incubation and its BOD is computed from the difference between the initial and final DO. The seeding and dilution procedures outlined in this method provide an estimate of the BOD at pH 6.5-7.5.

3.0 INTERFERENCES

A number of factors such as soluble versus particulate organics, settleable and floatable solids, oxidation of reduced iron and sulfur compounds or lack of mixing may affect the accuracy and precision of BOD measurements. There are no means at this time to include adjustments or corrections to account for these factors.
Nitrogenous demand has been considered as an interference in the determination of BOD which is prevented by the use of an inhibitory chemical. If an inhibiting chemical is not used then the oxygen demand measured is the sum of carbonaceous and nitrogenous demand.

4.0   SAFETY

When handling wastewater sample and using the acid and alkali for sample pH adjustment, protective gloves, clothing and eye-ware is to be worn.

5.0 EQUIPMENT AND SUPPLIES

5.1. Dissolved Oxygen Meter – YSI Multilab IDS 4010-2

5.2. Dissolved Oxygen Probe – YSI IDS ProOBOD Probe

5.3. 300 ml BOD Incubation bottles with flared necks and caps – KIMAX , Wheaton or Disposable Pre-cleaned PET resin incubation bottles from Environmental Express (Cat# D1001) equipped with Acrylic stoppers (Cat# D1025) and overcaps (Cat # D1050)

   5.3.1. Cleaning of BOD bottles & stoppers (glass – reusable)

   1. Soak bottles & stoppers in hot tap water containing detergent water for minimum 1 hour. Use a scrub brush to remove any remaining gunk. Rinse 3x with tap water. Let dry.
   2. Fill bottle half way with de-ionized water, add 10 mls of 10% HCL solution. Swirl. Fill the bottles completely with de-ionized water. Randomly measure the pH to assure that it is < 2 ph. Place stoppers into a beaker containing 10% HCL. Allow to sit for 24 hours.
   3. Triple rinse with de-ionized water. Let dry.

5.4. Carboys – 10 L & 20 L size for preparation of dilution water

5.5. Aerators – purchased from a Fish tank supply store

5.6. Magnetic stirrers, large stir bars

5.7. 600 mL beakers

5.8. Filtered compressed air (if DO de-saturation of samples is needed)

5.9. Incubators designed to maintain 20 ± 0.5 °C consistently for the incubation duration. Revco (Model # BOD50A14) and ThermoScientific Precision (Cat # 3975) are presently being used.

5.10 pH test strips (Cat #

5.11 Residual chlorine test strips (HACH Cat # 2745050 or # 2793944)

6 REAGENTS AND STANDARDS

6.1. Deionized Water – Millipore DirectQ UV3 (Mfgr# ZRQSVP030) capable of delivering > 18 mohms quality water. Or an equivalent water supply.

6.2. Dilution Water – The water to be used in the preparation of the sample dilutions can be either demineralized, distilled, tap or natural water that is free of biodegradable organics and bioinhibitory substances such as chlorine or heavy metals. For consistency – this lab uses water delivered from the Millipore system (above, Section 6.1).
Aerate the carboy containing the deionized water long enough (minimum of 2 hours) to achieve a DO saturation of at least 7.5 mg/L. The dilution water

The actual dilution water is prepared just before using and contains the following nutrients & minerals necessary for bacterial growth and. (Section 6.0.1.):

   6.2.1. Purchased – BOD Nutrient Buffer Pillows – depending on the amount of dilution water needed – choose the appropriate volume buffer pillow -
   HACH Cat# 1486166 - for preparing 3L

HACH Cat# 2436466 - for preparing 4L
HACH Cat# 1486266 - for preparing 6L
HACH Cat# 1486398 - for preparing 19L

6.2.2. Prepared - add 1ml of each of the following solutions to 1L of water

6.2.2.1.   Phosphate Buffer solution : Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4-7H_2O$, and 1.7 g NH_4Cl in 500 ml of distilled water and dilute to 1L. The pH should be 7.2 without further adjustment.

6.2.2.2.   Magnesium Sulfate solution : Dissolve 22.5 g $MgSO_4-7H_2O$ in distilled water and dilute to 1L.

6.2.2.3.   Calcium Chloride solution : Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1L.

6.2.2.4.   Ferric Chloride solution : Dissolve 0.25 g $FeCl_3-6H_2O$ in distilled water and dilute to 1L

6.3. Acid & Alkali solutions – for neutralization of caustic or acidic waste samples.

6.3.1.   1N H_2SO_4 – Slowly and while stirring – add 28 ml of sulfuric acid to distilled water and   dilute to 1L.

6.3.2.   1N NaOH- Dissolve 40 g sodium hydroxide in distilled water and dilute to 1L.

6.4.   Sodium Sulfite solution – for de-chlorinating samples with residual chlorine – Dissolve 1.575 g Na_2SO_3 in 1L distilled water. This solution is not stable so prepare daily, as needed.

6.5.   Nitrification Inhibitor – 2-chloro-6-(trichloromethyl) pyridine – supplied by HACH, Cat# 253335 (35g) for use in determining Carbonaceous Biochemical Oxygen Demand ($CBOD_5$)

6.6.   Glucose-Glutamic Acid Solution –

6.6.1   Prepared : Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 hr. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use.

6.6.2   Purchased : Environmental Express LabChem Inc. Cat# LC14845-1

6.7.   Seeding material – it is necessary to have present a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample. The source of seeding material can purchased or obtained from a wastewater source.

6.7.1. Purchased

6.7.1.1. PolySeed – Environmental Express # D1250

-4-

Preparation of Seed Inoculum - Place the contents of one Polyseed capsule into 500 ml of dilution water (discard the gelatin capsule). Stir, creating a vortex to aerate the seed solution, for 1 hour.

The "Bran" that is the carrier used for the microorganisms within the PolySeed capsules must be settled out of the PolySeed solution before using. Therefore, after rehydrating the PolySeed, allow 15 minutes for the bran to settle, then decant the supernatant carefully so to not to allow any bran in the biological solution into a clean 500 ml beaker with a sterile stir bar. (Option is to use a separatory funnel). Gently stir the PolySeed solution while using for the remainder of the test.

This solution should be used within 6 hours of rehydration.

6.7.1.2. PolySeed-NX – Environmental Express# D1255. To be used when determining Carbonaceous B.O.D. Preparation of PolySeed -NX Inoculum – see Section 6.6.1.1.

6.7.1.3. If purchased PolySeed is not available – then seeding material can be prepared from a wastewater source by using the supernatant from a domestic wastewater after settling at room temperature for at least one hour but no longer than 36 hours.

7.0   <u>SAMPLE COLLECTION, PRESERVATION AND STORAGE</u>

7.1.   Collect samples in a clean plastic or glass containers without any additives. All containers are either purchased pre-cleaned or are cleaned with detergent followed by a 3x wash with tap water and then a 3x wash with reagent water.

7.2.   The container size & the amount of sample collected will depend on the sample volumes required to provide three representative sample preparations which yield BOD values that fall within the acceptance criteria.

        General Rules for Sample Volumes
        Influent – 100 ml
        Effluent – 1000 ml

7.3.   Grab Samples :

7.3.1.   If analysis is begun within 2 hours of collection, cold storage is unnecessary.

7.3.2.   If analysis is not started within 2 hours of collection, the sample must be kept at $\leq 4.0\,^{\circ}$ C. Begin analysis within 6 hours of collection, when this is not possible, store at $\leq 4.0\,^{\circ}$ C and report length and temperature of storage with the results.

7.3.3.   In no case start the analysis more than 24 hrs after grab sample collection.

7.4.   Composite Samples :

7.4.1. Keep samples $\leq 4.0\,^{\circ}$ C during compositing. Limit composite period to 24 hrs.
Use the same storage criteria as for grab samples, starting the measurement of
holding time from end of compositing period. State storage time and conditions as
part of the results.
Warm chilled samples to $20 \pm 3^{\circ}C$ before analysis

7.5.   Sample Pretreatment

7.5.1. Check pH of all samples unless previous experience indicates that the pH is within the
acceptable range (6.0 – 8.0) using pH test strips.

7.5.1.1. Sample containing caustic alkalinity (pH >8.5) or acidity (pH <6.0) – neutralize
samples to pH 7.0 – 7.5 with a solution of sulfuric acid (H_2SO4) or sodium
hydroxide (NaOH) of such strength that the quantity used does not dilute the
sample by more than 0.5%. Always seed samples that have been pH adjusted.

7.5.3. Determine if samples contain any Residual Chlorine:

If possible, avoid samples containing residual chlorine by sampling before any
chlorination processes. If residual chlorine is present, dechlorinate sample.
In some samples – chlorine will dissipate within 1 to 2 hours of standing in light.

7.5.3.1. Testing for Residual Chlorine for chlorinated samples : Any treated samples will
need to be seeded.
Using a HACH residual chlorine test strips (HACH Cat # 2745050 or #
2793944) determine the level of free chlorine.

7.5.3.2. Destroy any residual chlorine by adding Na_2SO_3 solution (see Section 6.4)
Determine the required volume of Na_2SO_3 solution on a 100 mL neutralized
sample by adding 1 ml of 1 + 50 H2SO4 and 1 mL of potassium iodide(KI)
solution to sample and titrating to a starch-iodide end point for the residual
chlorine. Add to the neutralized sample the proportional volume of Na_2SO_3
determined by the above test, mix and after 10 to 20 minutes check for residual
chlorine.

7.5.4. Samples supersaturated with DO (> 9.0 mg DO /L at 20°C) – Reduce DO to saturation at
20°C by bringing sample to about 20°C in a partially filled bottle while agitating by
vigorous shaking or aerating with clean filtered compressed air

8.0 QUALITY CONTROL

8.1. Precision & Bias

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic acid check is intended to be a reference point for evaluation of the dilution water quality, seed effectiveness, and analytical technique.
The regression equations for the Mean value, X & and the standard deviation, S, from a series of interlaboratory studies are:

$$X = 0.658 \text{ (added level, mg/L)} + 0.280 \text{ mg/L}$$
$$S = 0.100 \text{ (added level, mg/L)} + 0.547 \text{ mg/L}$$

Therefore, for 300 mg/L mixed G/GA primary standard, the average 5 day BOD would be 198 $\pm$ 30.5 mg/L.
If the GGA results fall outside of this range – readjust the amount of seed used.

8.2. Working Range & Detection Limits

The working range is equal to the difference between the maximum initial DO (i.e. 7.0 – 9.0 mg/L) and the minimum DO residual of 1 mg/L multiplied by the dilution factor.

A lower detection limit of 2 mg/L is established by the requirement for a minimum DO depletion of 2 mg/L.

8.3. Control Limits

Establish Control limits by performing 25 Glucose-Glutamic Acid Checks over a period of several weeks and calculating the mean & standard deviation. Use the Mean $\pm$ 3 StdDev as the control limit for future G/GA checks.

9.0 CALIBRATION of the Dissolved Oxygen Meter – Water Saturated Air Method

a. Place the sensor in a water saturated air environment(BOD bottle with 40 mLof water Millipore). Leave the sensor om the calibration bottle 5-10 minutes, so that the air is water vapor saturated and the sensor cap can adapt to the ambient temperature. Turn power on.
b. Start the calibration with <CAL>.
c. Start the measurement with <ENTER>. The measurement value is checked for stability. The (AR) status indicator is displayed. The measurement parameter flashes.
d. Wait for AutoRead Measurement to be completed(audio signal). The calibration record is displayed.
e. Switch to the measure value display with <ENTER>. Read and record the DO's. After done, rinse the probe with Millipore water, shake off excess and place back in the BOD bottle with 40 mL of water. Shut off the power.
f. IDS DO sensors measure the temperature with a temperature sensor integrated in the IDS sensor.

10.0 PROCEDURE

a. Preparation of Blanks, Controls and Samples

1. Preparation of Dilution Water Blanks
    Fill two – three separate BOD bottles with dilution water alone.

2. Preparation of Seed Blanks
    Prepare three seed blanks using 10, 15, 20 mLs of the Polyseed solution and filling the BOD bottles with dilution water.

3. Preparation of Glucose /Glutamic Acid Check samples into three BOD bottles.
    Add a 6 ml aliquot of the Glucose /Glutamic Acid solution to each bottle containing 7 mLs of the Polyseed solution. Fill the rest of the bottle with the dilution water.

b. Preparation of Wastewater Samples

1. Determine the volumes of wastewater needed to achieve the estimated BOD values. Aim for dilutions that will result in a residual DO of at minimum 1 mg/L and a DO uptake of at least 2 mg/L after a 5 day incubation period.

    1a. If the estimated BOD value is known then these dilution volumes can be determined by the following calculations:

    Minimum Volume :
    mLs sample = 2 x 300 / estimated BOD

    Maximum Volume :
    mLs sample = 8 x 300 / estimated BOD

    1b. If the BOD of the waste water is not known, then an extended range of dilution aliquots will be necessary. Performing a COD test is an alternative guide is selecting dilutions.

    1c. If the estimated BOD value is very high, it may be difficult to accurately measure small volumes and get a representative sample. In such cases, an initial dilution should first be made on the sample. A 1:10 dilution is generally sufficient.

2. Add 7 mLs of the POLYSEED solution to the BOD that will contain dilutions of the sample that may have too few micro-organisms for the oxidation of the organic material in the sample.

3. If a nitrification inhibitor is used, add one shot (300 mg) of inhibitor to each dilution water blank, seed control blanks, Glucose/ Glutamic Acid checks and samples.

4. Set up at minimum three dilutions for each sample to cover estimated range of BOD values. Invert sample bottle 5 times to mix sample. Do not shake sample vigorously. Measure sample aliquots using wide-mouth tipped graduated pipets or graduated cylinders.

5. Fill the rest of the BOD bottles with enough dilution water so that insertion of the stopper will displace all air, leaving no bubbles. Tapping gently on the bottles with a metal rod helps in dislodging air bubbles.

6. Measure the initial DO of the dilutions for each sample run. If sample contains materials that react rapidly with DO, determine the initial DO immediately after filling the BOD bottle with the diluted sample. If the DO uptake is insignificant, then the time lapse between filling and measuring DO is not critical.

7. Stopper the bottle and place the plastic over- cap over the stopper. There should be a water seal formed around the stopper and the flared edge of the BOD bottle to reduce evaporation of water during incubation.

8. Incorporate a duplicate sample run for every 10 samples.

9. Incubate for 5 days $\pm$ 3 hours at 20 $^{\circ}$C $\pm$ 1 $^{\circ}$C.

10. Measure the Final DO of each sample at the end of the incubation period (recalibrate the O_2 probe prior to measuring the final DO)

11.0 CALCULATIONS, DATA ANALYSIS, and REPORTING

11.1. Calculations:

11.1.1. When the sample dilution is not seeded :

$$BOD_5, mg/L = (D1 - D2) \times \frac{(BOD \text{ Bottle Volume, mL})}{\text{Sample Volume, mL}}$$

11.1.2. When the sample dilution is seeded :

$$BOD_5, mg/L = (D1 - D2 - f) \times \frac{(BOD \text{ Bottle Volume, mL})}{\text{Sample Volume, mL}}$$

Where :
   D1 = Initial DO of diluted sample immediately after prep
   D2 = Final DO of diluted sample after 5 days of incubation
   f = Seed DO correction factor as determined from seeded blanks

Calculate Seed DO correction factor, f :
$$f = (B1 - B2) \times \frac{mL \text{ of seed in sample}}{mL \text{ of seed in controls}}$$

Compute the seed DO correction factor, f, for the 10, 15, 20 mL seeded blanks. Calculate the average and use this value to correct the DO uptake for the seeded sample dilutions

11.2. Data Analysis

Because the BOD test takes 5 days to complete due to the incubation period, quality control data can not be evaluated and any subsequent corrective measures can not be performed during the testing. If any of the following acceptance criteria can not be met then the test results are questionable. Fresh samples should be taken and the test repeated.

11.2.1. Dilution Water Blanks

The DO uptake of the dilution water after 5 days incubation at 20 $^\circ$C should not exceed 0.2 mg/L

11.2.2. Seed Blanks

The acceptance range of the seeding material is determine as follows:
The DO uptake per mL of seed added to each bottle using the ratio method by dividing the DO depletion by the volume of seed in mLs for each seed control bottle having at least 2.0 mg/L depletion and greater than 1.0 mgL minimum residual DO. Average the results. Seed dilutions showing widely varying depletions per mL (30 %) suggest the presence of toxic substances or large particulates in the seed suspension. If this happens, check or change the seed source.

11.2.3. Glucose / Glutamic Acid Check samples

For the Glucose / Glutamic Acid Check samples (GGA), the average 5-day BOD would be 198 $\pm$ 30.5 mg/L. Adjust amount of seed added to the GGA test to achieve results falling within this range.
The calculated BOD average for the Glucose / Glutamic Acid Check samples are charted and should be within the control limits as established using the Mean $\pm$ 3 standard deviations of 25 determinations of the check solution.
If measured BOD for a GGA check is outside the accepted control limit range, reject tests made with that seed and dilution water or identify such tests clearly in all data records and reports.

11.2.4. Wastewater Samples

The DO depletion of the wastewater samples should be at minimum 2 mg/L and have a minimum residual DO of 1.0 mg/L. For any of the dilutions that meet this criteria, the calculated BOD results can averaged and reported.

11.3. Reporting

11.3.1. Report results as mg/L BOD_5

11.3.2. If Nitrification is inhibited then report results as mg/L $CBOD_5$

12.0 POLLUTION PREVENTION & WASTE MANAGEMENT

The laboratory waste management practices are conducted consistent with all applicable rules and regulations as stated in the laboratory's *Sample and Waste Disposal* (Revision 003) on July 6, 2006.

Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

13.0 REFERENCES

13.1. American Public Health Association, American Water Works Association, and Water Environment Federation; 22nd Edition of Standard Methods, "Standard Methods for the Examination of Water and Wastewater", Method SM 5210B

Barnstable County Department of Health and the Environment Laboratory

SM 2320B

STANDARD OPERATING PROCEDURE

For

Determination of Alkalinity in Aqueous Samples

(Revision 007)

October 8, 2015

Signature   Date

Analyst: Yuankun Ni_____

Laboratory Director: Gongmin Lei   _____

Barnstable County Laboratory

STANDARD OPERATING PROCEDURE (SOP)
For

Determination of Alkalinity in Aqueous Samples

1.   SCOPE AND APPLICATION

    1.1   This method covers the determination of Alkalinity in drinking water, surface water, domestic water, industrial wastes, and other aqueous samples.

2.   SUMMARY OF METHOD

    2.1   An unaltered sample is titrated to an electrometrically determined end point of pH 4.5.   The sample must not be filtered or diluted.

3.   INTERFERENCES

    3.1   Substances, such as salts of weak organic and inorganic acids present in large amount, may cause interferences in the electrometric pH measurements.

    3.2   For samples having high concentrations of mineral acids, such as mine wastes and associated receiving waters, titrate to an electrometric endpoint of pH 3.9 Oil and grease, by coating the pH electrode, may also interfere, causing sluggish response.

4.   SAFETY

    4.1   Follow general laboratory safety guidelines, such as wearing safety glasses, gloves and a lab coat.

5.   EQUIPMENT AND SUPPLIES

5.1   PC Titrate (Man-Tech), Model PC Titration Plus

5.2   Gilson autosampler, Model 223

5.3   pH Electrode, Man-Tech, Model PCE 80-PH1013

5.4   50 mL plastic vials, Fisherbrand #05-539-9

5.5   Reference filling solution, 4M KCL, # R001013

5.6   Rinse solution, 200mL of pH 4.00 (SB101-4), Fisher Scientific, fill to 1000mL
         with tap water.

6.   REAGENTS AND STANDARDS

6.1   Reagent water – Deionized water from Milli-Q Direct 8/16 System or Millipore
        Direct-Q 3 System,

6.2   Buffer Solution pH 4.00 (Red), Fisher Scientific, # SB101-4
        Buffer Solution pH 7.00 (Yellow), Fisher Scientific, # SB107-4
        Buffer Solution pH 10.0 (Blue), Fisher Scientific, # SB 115-4
        Buffer Solution pH 7.00 (Clear), Fisher Scientific, # SB 108-1

6.3   0.02N Sulfuric Acid, Fisher Scientific SA 226-1or make from 98% H2So4(Fisher,
         #300-212)
6.4   Sodium Carbonate, Fisher Scientific S495-500

6   SAMPLE COLLECTION, PRESERVATION AND STORAGE

7.1   Samples are collected in 500 mL plastic bottles. All bottles must be thoroughly
        cleaned and rinsed with reagent water.

6.2   Samples are stored at 4°C until analysis but must at room temperature when
        analyzed. Do not open sample bottle before analysis.

6.3   Holding time is 14 days.

7   QUALITY CONTROL

8.1   A duplicate sample is to be included in any set of 10 samples. The agreement
        between the sample and the duplicate must be ± 20%. If it is outside these limits, a
        third sample is analyzed.   If this is outside the limits, a QC check is run to check

the calibration of the instrument itself. If the instrument is found to be out of calibration, it is to be recalibrated and all analyzes are repeated.

8.2   A Quality Control sample is to be run at the beginning. After every 10 samples, and at the end of the run, a CCV is to be run. Use pH yellow buffer (pH 7). The QC value is 25mg/L. It must fall within 10% of the range. If it is not within the range, a second analysis is performed to verify the measured value. Upon repeat failure, the results from this analysis will be considered unacceptable and the complete procedure will be repeated after the instrument is recalibrated.

>   8.2 1   To make the QC take 1.0g of Sodium Carbonate dilute to 1000mL with reagent water.
>   8.2 2   Take 25mL of the stock Sodium Carbonate solution and dilute to 1000mL for the QC Solution.
>   8.2 3   A low level standard is to be run on an ongoing basis. Take 5mL of the stock Sodium Carbonate solution and dilute to 1000mL.

8.3   A Method Blank is run immediately following every QC sample. For the Blank, take 40mL of reagent grade water and place it in a 50mL plastic vial, and put it on the autosampler.

8.4   Method Detection Limit (MDL)- An MDL is established using reagent water fortified at a concentration of three to five times the estimated detection limit. seven replicate aliquots of the fortified reagent water are run and processed through the entire analytical method. Then the MDL is calculated as follows:

MDL= (t) x (S $_{n-1}$)

Where,

t = student's value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.

S $_{n-1}$ = sample standard deviation (n-1) of the seven replicate analyses.

>   8.3.1   The laboratory conducts MDL study annually.   The MDL's must be run over a period of at least 3 days.

8.5 Accuracy and Precision- 4 replicates of a known value are prepared and analyzed. The mean measured concentration ($C_{\bar{x}}$) of the replicate values is calculated as follows

$$C_{\bar{x}} = \frac{(C_1 + C_2 + C_3 + C_4)}{n}$$

Where,

$C_{\bar{x}}$ = Mean recovered concentration of the replicate analysis.

$C_1, C_2, ...C_n$ = Recovered concentrations of the replicate 1,2, …n.

The value derived for $C_{\bar{x}}$ must be within $\pm$ 10% of the true value.

The percent relative standard deviation (%RSD) of the replicate analysis as stated above is calculated using the following equation.

$$\%RSD = \frac{(S_{n-1})x100}{(C_{\bar{x}})}$$

Where,

S_{n-1} = sample standard deviation (n-1) of the replicate analysis.
$C_{\bar{x}}$ = mean recovered concentration of the replicate analysis.

9   CALIBRATION

9.1   In a 50 mL sample vial, place 40 mL of each of the 3 buffers. In position #1 on The autosampler place the pH 4.00, in position #2, put in pH 7.00 (yellow), and in position #3, put in pH 10.0.

10.   OPERATIONAL PROCEDURE

10.1   Double click on PC Titration-

10.1 1   Main Menu will show up, Click on RUN Titration. Double Click on line 1, a menu will come up highlight pH CAL 4-7-10 and hit OK.
Under Order Number, highlight and click on Autogenerate.
Under Sample Name, type in pH cal
Under Vial#, type in #1
Hit Save As and type in the date, hit OK

10.1 2   Load the autosampler, #1 position is pH 4.00, # 2 is pH 7.00, and #3 is pH 10.0.

10.1 3   Hit START

10.1 4   The PC Titrate will perform the calibration and will say pH Calibration has passed.

10.2   SAMPLE ANALYSIS

10.2 1   Go to Titrator Main Menu- select RUN TITRATION

10.2 2   Double click on lst line- all the options will appear- select ALK QC-OK

10.2 3   Move curser to order#, hit AUTO GENERATE, move to sample name-type in ID of sample or QC, move to vial # and type that number in.

10.2 4   Go back to lst line-rt click- hit add a row or add rows
Double click on that next line, select pH QC-OK, move to order #, auto Generate, sample name, vial #,   Repeat these steps for all samples, Selecting the appropriate analysis –pH-Alk or Cond-pH-Alk options.

10.2 5   After all samples have been typed in and orders hit SAVE, type in current date, OK, then START.

11.   DATA ANALYSIS, CALCULATIONS AND REPORTS

11.1   Calculation:

The PC Titrate automatically calculates the titration.

11.2   Reports

All data, including the date, Lab ID, Client ID, the concentration (mg/L), and analyst initials are placed in the 3 ring binder PC Titrate logbook.

12.   POLLUTION PREVENTION AND WASTE MANAGEMENT

12.1   The laboratory waste management practices are conducted consistent with all Applicable rules and regulations as stated in the laboratory's Sample and Waste Disposal (Revision 003) on October 15, 2009. Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner in this SOP.

13.   REFERENCES

1.   Annual Book of ASTM Standards, Part 31, "Water", Standard D-1067, p 113 Method B (1976)

2.   American Public Health Association (APHA)
American Water Works Association (AWWA)
Water Environment Federation (WGI)
Standard Methods (SM) for the Examination of Water and Wastewater, 22nd edition SM2320B, 2012.

<u>YSI 556 Dissolved Oxygen Meter/ multimeter Calibrations</u>

Turn on meter and let rest for at least 20 min for DO sensor to polarize.

Check against pH 7, then DO, then Conductivity

Calibration Check Error Limits

Dissolved oxygen +/- 0.5 mg/l or +/- 6% saturation
pH   +-/ 0.15 standard units
Specific conductance +/- 5%

Calibrate in this order Conductivity, DO %, and then pH.

Store probe in either pH 4 buffers, or paper towel moistened with tap water.

Do Not Store in DI water.

<u>Calibration procedures</u>

Access calibration screen:

1. Press the escape key to display main menu
2. Use arrow keys to highlight the Calibrate section and press enter.

NOTE: Do not accept **OUT OF RANGE** on any parameter

If unit displays **OUT OF RANGE**
-Return to the calibration menu
-Select Parameter in question
-When prompted to enter value- **Press the Enter key and ESC key together simultaneously** (This will set the sensor back to factory default, erasing any erroneous information stored in the sensor)

YSI 556 Dissolved Oxygen Meter/ multimeter Calibrations

Conductivity Calibration

1. Select conductivity from Calibration menu, press enter.
2. Select Specific Conductance, press enter.
3. Enter Value of standard, 447 uS
4. Wait for reading to stabilize, press enter.
5. Unit is now calibrated.

Dissolved Oxygen Calibration

1. Return to calibration menu.
2. Select DO Calibration and press enter.
3. Select DO % and press enter.
4. Accept the barometric reading.
5. Fill cup with tap water, 3mm or 1/8" DO NOT SUBMERGE TEMP SENSOR.
6. Screw one or two threads of the transport cup to make sure sensors are vented to atmosphere.
7. <u>Allow at least ten minutes for the air in the cup to become water saturated and for the temperature to equilibrate before proceeding.</u>
8. When the reading under DO% stabilizes for at least 30 seconds press enter.
9. Press enter again and then escape to return to the calibrate menu.
10. Dry sensor

If the DO calibration gives OUT OF RANGE more than once the membrane should be changed.

-Do not touch membrane itself.
-Unscrew old membrane.
-Use sand disc to clean the DO electrode.
-Over fill new membrane with KCL solution.
-Screw on new cap, making sure there are no air bubbles.
-Recalibrate

<u>YSI 556 Dissolved Oxygen Meter/ multimeter Calibrations</u>

pH Calibrations

1. Return to Calibration menu.
2. Select pH press enter.
3. Select Calibration points you would like and press enter
4. Start with pH 7
5. Wait for reading to stabilize, press enter
6. Meter displays "calibrated" press enter to move to next pH
7. Repeat for 2 or 3 point calibrations

Once calibrations are completed, ESC to the RUN screen, and check calibrations verses accepted values

Record all calibrations in the calibration log sheet

Calibration Check Error Limits

Dissolved oxygen +/- 0.5 mg/l or +/- 6% saturation
pH   +-/ 0.15 standard units
Specific conductance +/- 5%

Appendix H:
Barnstable County Department of Health and the Environment Laboratory SM 9222 D
STANDARD OPERATING PROCEDURE for Determination of Fecal Coliform in
Aqueous Samples.

Barnstable County Department of Health and the Environment Laboratory

STANDARD OPERATING PROCEDURE

For

**Determination of Fecal Coliform in Potable and Non Potable Water Using
SM 9222D**

Revision (003)

December 7, 2016

		Signature	Date
Bacteriologist:	**Ryan Lucier**	_Ryan Lucier_ 12/16/16	
Laboratory Director:	**Gongmin Lei**	_Gongmin Lei_ 12/16/2016	

Table of Contents

STANDARD OPERATING PROCEDURE

For
Determination of Fecal Coliform in Potable and Non Potable Using
SM 9222D

1.0   Scope and Application

1.1   This method describes a membrane filter (MF) procedure and verification procedure for the detection and enumeration of Fecal Coliform in potable and non potable waters.

1.2   Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of levels in water can be detected and enumerated.

2.0   Summary of Method

2.1   The MF method provides a direct count of fecal coliform bacteria in water based on the development of colonies on the surface of the membrane filter. A water sample is filtered through the membrane which retains the bacteria. After filtration, the membrane containing the fecal coliform is placed on mFC agar and then placed in a water bath at 44.5±0.2°C for 24 ±2.0 hours.

3.0   Interferences and Contamination

3.1   Water samples containing colloidal or suspended particulate material can clog the membrane filter and prevent filtration, or cause spreading of bacterial colonies which could interfere with identification of target colonies.

4.0   Safety

4.1   The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials and while operating sterilization equipment.

5.0   Equipment and Supplies

5.1   Stereoscopic Microscope (vendor: Fisher Scientific, Stereomaster)
5.2   Hand tally or electronic counting device
5.3   Plastic or glass 1 and 10 mL graduated serological pipets
5.4   Graduated cylinders, 50 mL, 100mL, 250 mL, 500mL, and 1000mL
5.5   Ultraviolet light box for sanitization of stems and funnels

3

5.6   Electronic vacuum pump (Model # SKH33DN16GX, Vendor: VWR)

5.7   Flask, filter, with appropriate tubing

5.8   A five or six manifold filtration assembly (glass stems and funnels Vendor: Fisher)

5.9   Flat tipped forceps

5.10   Ethanol or isopropanol

5.11   Burner , Bunsen or Fisher type, or propane fuel lighter

5.12   National Institute of Standards and technology (NIST) certified thermometer (Thermometer, Cat. # 1005-3FC, range (-1+101°C, 1, 1°C)

5.13   Thermometers for incubators

5.14   Incubators (Vendor: VWR, Model: 1550 and 1545), Waterbaths (Vendor: Blue M, Model MW-1130A-1, and Vendor: VWR , Model 1265PC and 1203) with appropriate temperature range (35.5°, 41.0°, and 44.5°C)

5.15   Millipore membrane filters, sterile, white grid marked 0.45μm pore size 47mm diameter

5.16   Sterile Millipore petri dishes 9x50mm designed for 47mm filters

5.17   Flasks 100mL, 500mL, 1000mL, 2000mL

5.18   Plastic Inoculation loops at least 3mm diameter

5.19   Pyrex Borosilicate glass Test Tubes with autoclavable caps, size 20x 150mm

5.20   Whirl-Pak® bags

5.21   Hot plate (Vendor: Corning PC-620)

5.22   Stir bars

5.23   Safety glasses

5.24   Gloves

5.25   Lab coat

5.26   Balances (Vendor: Scout, Item # SC6010)

5.27   Autoclaves (Vendor: Tuttnauer Brinkmann, Model 3870M, Vendor: Market Forge, Model:STM-E)

6.0   Reagents and Standards

6.1   Purity of Reagents: Reagent-grade chemicals shall be used in all tests. Whenever possible, use commercial culture media as a means of quality control. Use Reagent grade water from the Milli-Q system to assure quality of water.

6.2   Phosphate Buffer Solution

      6.2.1   Purpose and Use:
            The phosphate buffer solution is used as a diluting and rinsing solution for the bacteria samples. The Magnesium Chloride is added to the buffer to increase the recovery of organisms with metabolic injury due to high quality waters or to waters containing significant concentrations of metals.

6.3   Preparation of Stock Magnesium Chloride Solution ($MgCl_2$):
Add 32.44g of Magnesium Chloride Solution ($MgCl_2 \cdot 6H_2O$) to 400mL
of reagent-Grade distilled or deionized water. Transfer to 500mL bottle
with a loose cap. Put autoclave tape on bottle and autoclave at 121°C for
15 minutes. Label bottle as "$MgCl_2$ Stock" with date and initials. Store
this in the refrigerator for future use.

6.4   Preparation of Stock Potassium Dihydrogen Phosphate Solution
(KH_2PO_4): Add 13.6g of Potassium Dihydrogen Phosphate to 200mL of
reagent-grade distilled or deionized water. Adjust pH to 7.2±0.5 with 1N
NaOH. Add remaining distilled water to bring to 400mL. Transfer to
500mL bottle with a loose cap. Put autoclave tape on bottle and autoclave
at 121°C for 15 minutes. Label as "KH_2PO_4 Stock" with date, pH and
initials.

6.5   Phosphate Buffer Solution Preparation

Composition:

Reagent-grade Distilled or Deionized water	2L
$MgCl_2$ Stock Solution	10mL
KH_2PO_4 Stock Solution	2.5mL

6.5.2   Preparation: Always take the initial pH of the KH_2PO_4 before
you prepare the buffer. The initial pH should be 7.2±0.5. Always
take the initial pH of the reagent grade distilled or deionized water
before making the buffer, the pH should be 5.5-7.5. Dissolve all
ingredients in 2L of Reagent-Grade distilled or deionized water by
stirring in a 2L flask. Stir and check pH. The final pH should be
7.2 ±0.2. If the pH is not within range, add 1-2 drops of 2N
NaOH; stir and check pH again. Keep adjusting pH until it is
within the range. Transfer buffer solution into 1L bottles filling
them only ¾ of the way full. Affix autoclave tape to caps and cap
bottles loosely. Autoclave the buffer for 30 minutes at 121°C.
Allow bottles to cool down before tightening caps. Date and initial
bottles and store at room temperature for future use. Perform
sterility checks on each batch of buffer before use (refer to section
8.18 in Quality Control section).

6.6   Tryptic Soy Broth(TSB) (Becton Dickenson:211825)

Composition:

Pancreatic Digest of Casein	17.0g
Enzymatic Digest of Soybean Meal	3.0g
Dextrose	2.5g
Sodium Chloride	5.0g
Dipotassium Phosphate	2.5g

6.6.1   Preparation: To prepare 1L measure out 30g of TSB using top loading balance. Suspend in 1L of reagent-grade deionized water in an Erlenmeyer flask. Warm slightly with stirring to dissolve completely. Add 25 mL of broth to 20, 30mL screw cap tubes. Before sterilization add 100mL of TSB to 5 clear 250mL bottles (for buffer sterility checks). Autoclave for 15 minutes at 121°C; do not exceed 45 minutes in autoclave. The final pH is 7.3±0.2 at 25°C. The TSB can be stored in the refrigerator for three months and then discarded.

6.7   R2A agar (Reference number: Becton Dickinson 218263)

Composition:

Yeast Extract	0.5 g
Proteose Peptone No.3	0.5 g
Casamino Acids	0.5 g
Dextrose	0.5 g
Soluble Starch	0.5 g
Sodium Pyruvate	0.3 g
Dipotassium Phosphate	0.3 g
Magnesium Sulfate	0.05 g
Agar	15.0 g

6.7.1   Preparation: To prepare one liter of media suspend 18.2 g R2A agar in one liter of reagent grade deionized water in a 2 liter Erlenmeyer flask. Heat to boiling with stirring until completely dissolves. Be careful that the media does not burn on the bottom of the Erlenmeyer flask. Dispense in 12 ml amounts after tempering into 16 x 150 mm screw cap tubes. The media can last up to 3 months being stored at 4°. Autoclave agar for 15 minutes at 121°C. Do not exceed 45 minutes in autoclave. Final pH should be 7.2±0.2 at 25.0°C.

6.8   mFC agar (Reference #: Becton Dickinson 267720)

Composition:

Pancreatic Digest of Casein	6.0g
Proteose Peptone No.3	9.0g
Yeast Extract	3.0g
Lactose	12.5g
Bile Salts No. 3	1.5g
Sodium Chloride	5.0g
Agar	15.0g
Aniline Blue	0.1g

6.8.1 Preparation: This media cannot be autoclaved. All utensils and containers must be sterilized prior to use. To make 1L of media, prepare 10mL of 1% Rosolic acid solution. Add 0.1g Rosolic acid (cat. # 83910) and dissolve in 9.9mL 0.2 NaOH. Store at 4° C in a screw cap tube for up to two weeks; discard if solution becomes brown or muddy. Measure 1 L of reagent-grade deionized water in a graduated cylinder and pour into a 2 liter Erlenmeyer flask containing a stir bar. Add 52g of mFC agar to the flask. Boil to dissolve completely. Add 10mL of a 1% solution of Rosolic acid in 0.2N NaOH and continue heating for 1 minute. The final pH should be 7.4±0.2 at 25 °C, adjust with 1N HCL if necessary. Dispense 4-5 mL of tempered media (45° C) into sterile petri dishes. Allow to solidify and invert and store in labeled box at 4°C. Media can last up to two weeks.

6.9   EC Medium (Reference #: Becton Dickinson 231420)

Composition:

Tryptose	20.0g
Lactose	5.0g
Bile Salts No. 3	1.5g
Dipotassium Phosphate	4.0g
Monopotassium Phosphate	1.5g
Sodium Chloride	5.0 g

6.9.1 Preparation of EC Medium: To prepare 400 mL of broth (makes approximately 40 tubes). Add 14.8g of EC Medium in 400mL of deionized water in a sterile 600mL beaker. Warm the broth slightly with stirring to dissolve agar completely. Use Durham tubes for EC media. Place test tubes into test tube racks that can withstand the autoclave. Place an inverted Durham fermentation tube into each test tube. Dispense 10mL amounts into each test

tube. Cap loosely, affix autoclave tape onto tubes. Sterilize in the autoclave for 15 minutes at 15psi and 121°C. Limit the time in the autoclave to 45 minutes. Before opening autoclave, allow the temperature to drop below 75° C to avoid entrapping air bubbles in the inverted tubes. After the tubes have cooled, tighten the caps of the tubes and check to see that no air bubbles are present in the Durham tubes. If air bubbles are present in the Durham tubes, the media must be discarded. The final pH when cooled to 25°C should be 6.9±0.2. Label the rack with date, type of media, and initials. Media should be stored in a dark cabinet at room temperature to be used within 3 months.

6.10   Laurly Tryptose Broth (LTB) (Becton, Dickinson and Company; Reference # 224140)

Composition:

Pancreatic Digest of Casein	12.0g
Proteose Peptone No. 3	8.0g
Lactose	5.0g
Dipotassium Phosphate	2.75g
Monopotassium Phospate	2.75g
Sodium Chloride	5.0g
Sodium Lauryl Sulfate	0.1g

6.10.1 Preparation of LTB: to prepare 400mL of LTB (makes approximately 40 tubes) add 14.2g of LTB in 400mL of reagent-grade deionized water in a sterile 600mL beaker. Dissolve broth completely by heating and stirring but do not allow it to boil. Use Durham tubes for LTB. Place test tubes into test tube racks that can withstand the autoclave. Place an inverted Durham fermentation tube into each test tube. Dispense 10mL amounts into each test tube. Cap loosely, affix autoclave tape onto tubes. Sterilize in the autoclave at 121°C and 15psi for 15 minutes. Limit the time in the autoclave to 45 minutes. Before opening autoclave, allow the temperature to drop below 75° C to avoid entrapping air bubbles in the inverted tubes. After the tubes have cooled, tighten the caps of the tubes and check to see that no air bubbles are present in the Durham tubes. If air bubbles are present in the Durham tubes, the media must be discarded. The final pH when cooled to 25°C should be 6.8±0.2. Label the rack with date, type of media, and initials. Media should be stored in a dark cabinet at room temperature and needs to be used within 3 months.

6.11   Holding Times for Media:

Media	Holding time
MF agar in plates with tight-fitting lids in refrigerator	2 weeks
Agar or broth in tightly closed screw-capped tubes in refrigerator	3 months
Poured agar plates with loose fitting covers in sealed plastic bags in refrigerator.	2 weeks
Agar or broth in loose-cap tubes at 4° C	1 week
Dehydrated Media opened in dessicator	1 year from date opened
Dehydrated Media opened not in dessicator	6 months from date opened

7.0   Preservation and Storage

7.1   Start microbiological analysis of water samples as soon as possible after collection to avoid unpredictable changes in the microbial population. For most accurate results, ice samples during transport to the laboratory and do not submerge samples in water or ice during transport to the laboratory. This could cause cross contamination in the samples.

7.2   Drinking water for compliance purposes: Preferably hold samples <10°C during transit to the laboratory. Analyze samples on day of receipt whenever possible and refrigerate overnight if arrival is too late for processing on same day. Do not exceed 30 hour holding time from collection on analysis for coliform bacteria.

7.3   Nonpotable water for compliance purposes: Hold source water, stream pollution, recreational water, and wastewater samples below 10°C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours. When transport conditions necessitate delays in delivery of samples longer than 6 hours,

consider using either field laboratory facilities located at the site of collection or delayed incubation procedures.

7.4 Other water types for noncompliance purposes: Hold samples below 10°C during transport and until time of analysis. Do not exceed 24h holding time.

8.0 Quality Control

8.1 Thermometers and temperature recording equipment- The accuracy of each thermometer is checked annually against a certified NIST thermometer at the temperature used. In general the graduations on the thermometer should be in increments of 0.5 degrees or less. The maximum thermometers and NIST thermometers are checked annually by Alert Scientific. The result of each thermometer check is recorded in the thermometer logbook.

8.2 Balances- Balances are checked daily with the provided class S weights. The top loading balance is for weighing 3g or more. For this balance the 100.0g, 50.0g, 1.0g, and 100mg weights are used. Each weight is weighed independently, and in addition, a deflection test is performed prior to usage. The results of the check are recorded in the assigned balance book. The S class weights and balance are checked annually by Alert Scientific.

8.3 pH Meter- The pH meter is to be calibrated daily with at least three standards: 4.00, 7.00, and 10.00. The standards need to be changed daily. The temperature, slope, and pH are recorded in the temperature logbook. The pH meter is checked annually by Alert Scientific.

8.4 Media dispensing apparatus- Check accuracy of volume by pipetting the amount into a weighing dish on the top loader balance. Adjust the setting accordingly. When sterilizing, put the glass barrel in the apparatus and tighten finger tight and then loosen quarter of a turn, this lets airflow through the setup so the pressure, from autoclaving, won't build up and break the glass. Also, fill an Erlenmeyer flask with reagent grade water and sterilize. This water is used to pump through the syringe at the end of each use. If there is any visible staining at the end of use, boil in reagent grade water.

8.5 Autoclave (Tuttnauer Brinkmann Model 3870M and Market Forge Model # STM-E)- Record items being sterilized, temperature (maximum thermometer), pressure and time for each run. Use BTSure ampoules (catalog # AY759X3) weekly by placing the ampoule in the autoclave for 15 or 20 minutes at or above 121°C. After autoclaving is complete, let ampoule cool and then gently squeeze and break the glass ampoule. The

ampoule will now be activated. It must immediately be placed in a screw cap tube and placed in the small water bath at 55°C for 48 hours. Also activate and incubate one ampoule that has not been autoclaved as a control. The control will turn yellow after incubation and if there is no color change (ampoule remains purple) for the autoclaved ampoules then it is concluded that the sterilization process of the autoclave is working properly. Make sure there is enough water in the water bath so BT Sure ampoules are completely submerged throughout the incubation period. Autoclave tape is used on every sample bottle before autoclaving. A maximum temperature thermometer is used and temperature is recorded after each run. The Autoclaves are checked annually by Alert Scientific.

8.6   Refrigerators- Check and record all temperatures daily. Use a refrigerator maintaining a temperature of 1 to 4.4°C to store samples, media, reagents, etc.

8.7   Water baths-   Temperature is checked and recorded twice daily with a four hour separation of time between readings and adjusted when needed. The time of the temperature check will also be recorded.

8.8   Incubators-   Temperature is checked and recorded twice daily with a four hour separation of time between readings and adjusted when needed. The time of the temperature check is also recorded.

8.9   Membrane filter equipment-   Wrap filter units in sterile wrap and autoclave daily for 20 minutes at 121°C. Before each set of samples expose stems and funnels to 2 minutes of UV light. Wash filter units weekly with detergent and rinse with deionized water before the daily autoclaving. Record the lot number and date of receipt of new Millipore membrane filters in the microbiology primary standard logbook when they are received.

8.10   Ultraviolet sterilization lamps- Clean lamps with ethanol monthly and record in quality control logbook. Do a sterility check to assure proper sterilization. Either stock organisms or a known positive water source can be used.

8.10.1 Procedure for UV sterility check- Label R2A plates as follows:
1. Control (swabbed)
2. UV left
3. UV center
4. UV right

Pouring Plates: Melt solid R2A agar (refer to section 6.7 for media preparation) in boiling water and put one tube of water or an extra R2A tube in the boiling water and then transfer to 46°C water bath.

Place a thermometer in the water tube or extra R2A tube to indicate medium temperature. Melted agar must be used within three hours and can not be re-melted. Agar can be used when temp cools to between 44-46°C. Allow no more than 20 minutes to elapse between plating sample and pouring medium. Before pouring agar wipe the tube dry with a paper towel and flame the neck of the tube. Carefully pour the contents of one tube (12mL) into the 100x15mm petri dish, and mix contents by rotating clockwise and then counter clockwise to cover the bottom surface of the petri dish. Allow agar to solidify. Pipet 0.1mL or a five-fold dilution of the broth culture or 0.1mL of a two-fold dilution of the raw sewage sample onto each of the five plates, using a sterile glass spreader, spread the inoculums evenly over the entire surface of the agar of each plate. Plate #1 is to be left out of the UV box as the control. Remove the lids of plates 2-4; place the plate into the light box. Set the timer to two minutes and turn on the UV lights. At the end of two minutes turn off the UV lights and remove the plates. Incubate the UV sterilized plates and the controls for 48 hours at 35±0.5° C. After the incubation period check plates for growth, control plates should show heavy growth, (>200 colonies) and sterilized plates should show little to no growth (0-2CFU). The kill rate from the lamps should be 98%. If more than 2 colonies form on a plate then lamps need replacing.

8.11   Milli-Q deionized water - The sterility of reagent water should be checked monthly using pour plate method with R2A.

8.11.1 Sterilit y Procedure: 1mL of the Milli-Q water is pipetted into a large sterile petri dish. Sample is run in duplicate on another plate. Heat tempered R2A (refer to section 6.7 for media preparation) is poured over sample and swirled clockwise and counterclockwise. After the plates solidify they are inverted and incubated for 48 hours at 35.0 ± 0.5°C. After the incubation period check plates for growth; this water should be sterile.

8.12   The Total Chlorine residual of the reagent water is checked monthly.

8.12.1 Total Chlorine Procedure :   Using chlorimeter fill one test cell with reagent water to 10mL line. Add contents of one DPD Total Chlorine pouch to the sample cell; cap and shake for 20 seconds. Set timer for 3 minutes; during this time fill the other cell with 10mL of the reagent water and cap (this is the blank). Place blank in chlorimeter with cover on and press ZERO. Remove blank cell and within 3 minutes after the 3 minute period place prepared sample cell in chlorimeter, cover, and press READ. Record result in QC Log (result must be less than 0.1mg/l).

8.13   The pH of the reagent water is checked before each use. Fill clean beaker with reagent water and stir. Measure the pH with a calibrated meter. Record result in media preparation log with the media (pH of water must be 5.5-7.5).

8.14   Air Quality-   The air quality in the laboratory is monitored monthly. Plates of R2A media are left open in different parts of the laboratory for 15 minutes. A sterile plate is also poured and left closed for the 15 minutes. Incubate plates @ 35±0.5°C for 48 hours and observe for growth and record in appropriate book.

8.15   Glassware-   Glassware must have the pH checked because some cleaning solutions are difficult to remove completely, spot check batches of clean glassware for pH reaction, especially if soaked in alkali or acid. This is done by adding a few drops of 0.04% bromthymol blue (BTB) to a bottle and observing for a blue-green color indicating neutral pH. One of the bottles from each batch of washed and dried bottles needs to be tested by adding 3 drops of 0.04% BTB to the bottle. Observe for any color reaction. If the color is blue-green then the bottle from the batch can be used. If the color is yellow (acid) or blue (basic) then that batch would need to be rewashed. The results are to be recorded in the QA/QC log. If 0.04% of bromthymol blue needs to be prepared: add 16 mL 0.01N NaOH to 0.1g BTB and dilute to 250mL with distilled water.

8.16   All Microbiological bottles need to be autoclaved. Non-disposable sample bottles are washed in the dishwashers (using a distilled water rinse cycle).Bottles and caps are then removed from the dishwasher. All caps need heat activated autoclave tape affix on them and then they are placed in a clean beaker and covered with aluminum foil. Bottles and all caps are autoclaved for 20 minutes at 121-134°C. Make sure the tape has turned to a black color. When handling caps after being autoclaved use aseptic techniques when taking them out of the beaker and tightening caps on bottles when removed from the autoclave and cooled.

8.17   Autoclaved and sterile bottles need a sterility check. For each new batch of bottles received and at least one batch of autoclaved bottles per day perform a sterility check by adding approximately 25mL of sterile non selective broth (Tryptic Soy Broth) to at least one bottle. Cap bottle and rotate so that broth comes into contact with all surfaces. Check for any growth after incubation at 35°C at 24 hours and at 48hours. Record results in Quality Control Log. If TSB does not remain clear rewash and autoclave all bottles from the batch/batches in question.

8.18   Buffer Sterility Checks- The sterility of the buffer needs to be checked after it has been autoclaved and about to be put away for use. After the

13

Tryptic Soy Broth has been made according to TSB preparation in section 6.6, add 20mL of prepared buffer water to a TSB bottle using a top loader balance. Once combined shake the bottle and mix the buffer with the TSB. Label the bottle with the date and time it enters the incubator with the tech's initials. Incubate at 35°C checking for any growth and/or cloudiness at 24 hours and at 48 hours. If solution appears cloudy it was not sterilized properly and the batch must then be re-sterilized. If the TSB is clear then buffer can be put away for future use in cabinet. This information is then documented in the QC logbook.

8.19   Utensils and containers for media preparation- Only use utensils and containers of borosilicate glass, stainless steel, aluminum, or other corrosion-resistant material. Do not use copper utensils. Use glassware that is clean and free of residues, dried agar, or other foreign materials that may contaminate media.

8.20   Culture Media- Culture methods depend on properly prepared media; use the best available materials and techniques in media preparation, storage, and application. Order media in quantities that last no longer than a year. When media is received record kind, amount, lot number, expiration date and date received. Store media in a cool and dry place away from sunlight. Store opened containers of media in a desiccator immediately after use. Discard media that cake, discolor, or show other signs of deterioration. If expiration date is given by manufacturer, discard unused media after that date. Discard any opened media after one year. Never combine different lots of media.

8.21   New Lot testing- Pure culture comparison testing and positive and negative controls need to be performed whenever a new lot of culture media is opened.
Batch testing- Each batch of laboratory-prepared media must be tested prior to use with at least one pure culture control of a positive and one pure culture of a negative (non-target reaction, as appropriate to method) reaction.

Target Bacteria	Positive Control	Negative Control
Fecal coliforms	*Escherichia coli*	*E. aerogenes* *E. faecalis*

8.22   Comparative Testing Procedure: Use the old batch as your control group. Make parallel tests between the control group and the test group. As a minimum make a single analysis using five pure positive control stock organism samples (Example: E.coli). After incubation, compare bacterial colonies from the two lots for size and appearance. If colonies on the test lot plates are atypical or noticeably smaller than colonies on the reference lot plates, record the evidence of inhibition or other problem, regardless of

14

count differences. Re-test media and if the differences still occur discard media.

    8.22.1 Positive and negative controls: Using known organisms test each new lot of media to ensure proper growth. Swab new media plates or tubes with appropriate control cultures and incubate at appropriate time and temperatures. Record results in Quality Control logbook.

8.23   For routine performance evaluation, repeat counts on one or more positive samples at least monthly and compare the counts with those of other analysts testing the same samples. Replicate counts for the same analyst should agree within 5% and those between analysts should agree within 10%.

8.24   Analysts must perform analysis of samples for various parameters in a uniform fashion. Example: If a sample needs fecal coliform, total coliform and E.coli run, then the analyst will perform all three tests with appropriate dilutions first before moving on to next sample.

8.25   Precision of Duplicate Analysis: Duplicates are run on 10% of samples for all analyses requiring enumeration. For each most recent set of 15 samples and its corresponding duplicate (for each type of sample analysis **i.e. fecal, e.coli, enterococci**), the range of logs is calculated in order to determine the acceptance criteria of precision. Once established, the criteria of precision will be used to determine if the following duplicate analyses are within acceptable limits. At the end of each week the criteria are updated to include the most recent set of 15 samples run in duplicate. The updated precision is used to determine precision acceptability for the following week.

8.25.1 Obtain the recorded numbers of the most recent 15 samples run in duplicate (for the same type of analysis).

8.25.2 Calculate the logarithm of each result (and if any result is <1 then add 1 to both values before calculating the logarithm) and record in a spreadsheet as *L1* and *L2*.

8.25.3 Range of Logarithms (R_{log}) is calculated using the following equation:

$$R_{log} = |\ L1\text{-}L2\ | \qquad\qquad (1)$$

8.25.4 The mean ($\bar{R}$) of R_{log} is calculated as follows:

$$\bar{R} = \frac{\sum R_{log}}{n} \qquad\qquad (2)$$

15

$\sum R_{\log}$ = The sum of the range of logs.

n = The number of sets of transformed duplicates.

8.25.5 Precision Criterion is cal culated as follows:

$$Precision\ Criterion = 3.27 \times \overline{R} \qquad (3)$$

8.25.6 An y samples run in duplicate (10% of all samples) have their Range of Logarithms ($R_{\log}$). If the range is greater than the Precision Criterion, there is a greater than 99% probability that the analysis has exceeded variability limits. For any samples that fall outside the acceptable limits, determine if the imprecision is acceptable. If data is not acceptable, all results since the last precision check should be rejected. The analytical problem(s) should be determined and corrective action should be taken to resolve problem.

8.25.6 See **Appendix A** for an example of the precision criterion.

9.0   Procedure

9.1   Procedure for membrane filtration:

9.1.1   Analyze samples within 8 hours of collection unless customer specifies otherwise. Samples must be preserved in a refrigerator or cooler until analysis.

9.1.2   Use mFC agar for fecal coliform identification.

9.1.3   Sterilize work area by washing down with a solution of 50% Bleach, 2%Lysol, or Conflikt.

9.1.4   CAUTION: UV light is carcinogenic and damaging to eyes. Sterilize filter funnel and bases in UV light box for 2 minutes. Turn light off before lifting wooden cover. Daily wrap the filter funnels in steriwrap and sterilize in autoclave for 20 minutes at 121°C.

9.1.5   Label plates on the bottom (media side) with bottle # and dilution if other than 100mL of sample is used. Record time on each plate.

9.1.6   Place the filter funnel base in manifold. Be sure valve is open.

9.1.7   Dip the forceps in alcohol (95% ETOH) and pass through the flame.

9.1.8   Using aseptic technique, remove sterile white gridded 0.45μm (pore size) 47mm (diameter) Millipore membrane filter with flat forceps from package touching only 1/8" of the filter's outside perimeter.

9.1.9   Place filter on base.

9.1.10 Place the funnel on the base.

9.1.11 Shake sample 25 to 30 times vigorously immediately before pouring.

9.1.12 Place the appropriate amount of sample into funnel:

> 9.1.12.1   When the sample volume used for dilution ranges from 1.0 mL to 10 mL, add 10-30mL of sterile buffered water to the funnel, and using disposable graduated pipet to transfer certain amount of the samples (1.0 mL-10.0 mL) to the funnel.

> 9.1.12.2   When the sample volume used for dilution is less than 1.0 mL, dilutions will be conducted in sterile bottles and then transfer diluted samples from the bottles to the funnels containing 10-30 mL sterile buffer solution.
>
> For additional dilutions you will need to make the dilution bottles. The dilution bottles can be filled as follows: For 10^{-3} and 10^{-4} dilutions you need 1 dilution bottle ; for 10^{-5} and 10^{-6} dilutions you need 2 dilution bottles (for **each** sample that will require either (or both) of these dilutions). (This is due to the fact you will still need to create a 10^{-3} and 10^{-4} dilution bottle in order to dilute the original sample properly and then dilute again to get an accurate 10^{-5} and 10^{-6} dilution).
>
> To create dilution bottles obtain sterile , non-thio Idexx bottles. Fill each of these to 99.0mL with sterile, autoclaved, tested buffer. The dilution bottle is now ready to be spiked with the sample in order to obtain the proper "dilutions" that you need.
>
> Line all of the dilution bottles along with the original sample. Label dilution bottles with sample

number and name, date, bacteria to be analyzed, and dilution(s). Set out 1mL pipettes to deliver sample into each bottle. Each dilution bottle requires 1mL of that sample which precedes it. For example, for the first original sample you will shake the bottle vigorously and then deliver 1mL into the first dilution bottle to achieve a sample bottle that represents the dilutions 10^{-3} and 10^{-4}. You will then set your first original sample bottle aside because you no longer need it for this portion. To dilute further, you will take the 10^{-3} and 10^{-4} dilution bottle, shake it vigorously and take 1mL from the 10^{-3} and 10^{-4} dilution bottle and add it to the 10^{-5} and 10^{-6} dilution bottle which will be the next in line. You are now able to test each dilution. Continue in this manner for **each** sample that requires dilutions.

9.1.13 For drinking water samples, shake sample and pour 100mL of sample into funnel. Sample volume is measured by the line on the funnels that has been calibrated using a 100mL volumetric flask. For non potable water sources use appropriate volume.

9.1.14 Turn vacuum pump on (Millipore Vacuum/Pressure Pump), after sample has filtered through, rinse funnels 3 times with approximately 20-30 mL of sterile buffered water.

9.1.15 Run duplicate bacteria analysis on 10% of all samples known to give positive results.

9.1.16 Using sterile flat forceps remove filter from the filter base again being careful to touch only the outside edge of filter. Place the filter on an mFC plate and make sure there are no air spaces between filter and agar surface (the filter can be picked up and reseated if necessary). Place plates in water bath bag (stacked up to three plates high maximum) and close bag so that it is watertight and place in water for 24 hours ± 2 hours at 44.5±0.2°C so the media side of the plates is on top. The media side of the plates has to be on top, so if moisture builds up in the petri dish it will not collect on the filter side and interfere with bacterial growth. Plates must be placed into water bath within 30 minutes after membrane filtration procedure. Make sure samples are submerged under water at all times by placing samples under the water bath rack and then place bricks or weights on top of the water bath rack.

9.1.17 Sterile controls are run by filtering 100mL of buffered water at the beginning and end of each series, when a different type of media is

being used and when switching between water sources (sewage to surface waters). Label the plates as "Sterile" and the time.

9.1.18 Examine plates after the required incubation using the stereo microscope with light source at 10-20 X magnification. Count all blue colonies.

9.2 Verification of Fecal coliform:

9.2.1 Verify colonies monthly (year round) from a known positive source.

9.2.2 Pick at least 10 blue colonies from membranes on mFC medium and transfer to separate Lauryl Tryptose Broth (LTB) tubes and incubate at 35° C checking for gas production at 24 and 48 hours.

9.2.3 Transfer growth from positive LTB tubes to EC broth and incubate in water bath at 44.5°C for 24 hours.

9.2.4 Growth and gas in EC broth tubes verifies colony as fecal coliform.

9.2.5 To determine false negatives pick atypical colonies and verify as above.

10.0 Data Analysis and Calculations

10.1 General information

10.1.1 Record all results in appropriate logbook and the chain of custody on the same day as sample analysis.

10.1.2 Record date, time of analysis and incubation, media, dilution, result, and analysts initials for all samples and steriles.

10.1.3 If a plate is run in duplicate, only use the plate that is not the duplicate. Use the duplicate results for QC data.

10.1.4 If there are any mistakes when writing in any log book cross out error with a single line and initial and date the change.

10.1.5 Enter results into the LIMS for report processing.

10.2   Calculation of Colony Density

10.2.1 Compute the count, using membrane filters with colony counts within the ideal range (fecal coliform:20-60 colonies per 100 mL), by the following equation:

Colonies/100mL = $\dfrac{\text{colonies counted} \times 100}{\text{mL sample filtered}}$

10.2.2 If no filter has a plate falling in the ideal range, compute the count using the following equation:

Colonies/ 100 mL = $\dfrac{[(\text{Sum of colonies counted on each plate}) \times 100]}{(\text{Sum of each volume analyzed in mL})}$

Example: If 50, 25, and 10-mL portions were examined and the counts were 15, 6, and <1 colony, respectively, follow the equation:

$$\dfrac{[(15 + 6 + 0) \times 100]}{(50 + 25 + 10)} = 25\, colonies / 100\, mL$$

10.2.3 Follow the table below to calculate dilutions using 100mL: Anything under 1mL will need a dilution prepared.

10.2.4 Use the excel spre adsheet when calculating the colony densities. When more than one dilution gives a zero result, use only the first zero result (this will correspond to the highest volume of sample with no growth.). Even when a duplicate is run on a sample and both results are zero, still use only one zero in the final calculation.

Dilution Factor	Volume of Sample (mL)
10	10.0
10^2	1.0
10^3	0.1
10^4	0.01
10^5	0.001
10^6	0.0001
10^7	0.00001
10^8	0.000001

10.2.5 If plates have distinguished recognizable colonies, but seem to have greater than the ideal counting range, try and count the plate. Use the number in the above equation. If confluent growth occurs, covering either the filtration area of the membrane or a portion thereof, and colonies are not discrete, report results as "too

numerous to count" (TNTC). These results will not be included in the equation above.

10.3 Data Reporting Limits (RL): All the final data are entered manually into the laboratory Information Management System (LIMS) and printed out through the LIMS. The reporting limits on the reports are calculated based on the sample dilution. Factors as follows:

10.3.1 If the sample is needed for dilution and more than one dilution are needed for calculating the final concentration, the smallest dilution factor will be used for the RL. The dilution factor can be calculated using the following equation:

$$Dilution\ Factor = \frac{100\,(mL)}{Sample\ Amount\ Taken\ for\ Dilution\,(mL)}$$

For example:
100mL and 10mL were taken and the final result was obtained based on calculation of the two individual results. Then RL= 0 CFU/100mL

1mL, 0.1 mL and 0.01 mL were used and the final result was obtained based on calculation of the three individual results.
Then RL= 100 CFU / 100mL

11.0 Disposal of Wastes

11.1 Biological wastes, such as petri dishes with bacterial growth, cotton-tipped applicators swabbed with bacteria, and confirmation and/or verification test tubes, shall be autoclaved at 121°C for thirty minutes and disposed of with standard trash. Little chemical waste is generated in association with the microbiological analyses. Those chemical wastes generated shall be stored in a designated area and segregated from non-compatible wastes and removed annually by a licensed Hazardous Materials Disposal Company.

12.0 References

12.1 American Public Health Association, American Water Works Association, Water Environment Federation; Standard Methods, "Examination of Water and Wastewater', Revision 20th Edition, 1998, Methods Quality Assurance /Quality Control 9020; Laboratory Apparatus9030; Preparation of Culture Media 9050; Samples 9060; Membrane filter technique for members of the coliform group 9222, 9222D, 9222B; Membrane Filter Techniques 9230C

13.0 Appendix A- Precision Criterion Table	mTEC- *Escherichia coli*	Duplicate Analyses		Logarithms of Counts		Range of Logarithms (R_{log})
Sample No.	Analytical Date	D1	D2	L1	L2	$\|L1-L2\|$
1	50031-11/14/08	4	1	0.60206	0	0.60206
2	50017- 11/13/08	12	10	1.079181	1	0.079181
3	49928- 11/4/08	9	5	0.954243	0.69897	0.255273
4	49886- 10/30/08*	1	1	0	0	0
5	49863-10/29/08**	1	1	0	0	0
6	49814- 10/23/08	20	23	1.30103	1.361728	0.060698
7	49764- 10/21/08	5	5	0.69897	0.69897	0
8	49728- 10/16/08	6	7	0.778151	0.845098	0.066947
9	49666- 10/14/08	1	2	0	0.30103	0.30103
10	49591- 10/7/08	7	6	0.845098	0.778151	0.066947
11	49557- 10/2/08	11	10	1.041393	1	0.041393
12	49512- 9/30/08	10	7	1	0.845098	0.154902
13	49512- 9/30/08	2	2	0.30103	0.30103	0
14	49456- 9/25/08	13	17	1.113943	1.230449	0.116506
15	49402- 9/23/08***	1	1	0	0	0

1) $\Sigma R_{log} =$ 1.744935

2) $\overline{R} = \dfrac{\Sigma R_{log}}{n} =$ 0.116329

Precision criterion

3)   = 0.380396

*= Value of D2 is 0
**=Value of D1 and D2 is 0
***=Value of D1 is 0

23